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8304 P*

### Incidence of Mammary Cancer and Nature of the Sexual Cycle in Various Strains of Mice.†

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Loeb and Genther[1] studied the characteristics of the sexual cycle in 2 different strains of mice, which were believed to differ in their incidence of mammary cancer. Subsequently it became doubtful to what degree the cancer incidence differed in these mice, and furthermore a comparison of only 2 strains of mice did not seem sufficient. Soon after completion of this first series, we began therefore a new and more extensive series of experiments. In the meantime there

* P represents a preliminary, C a complete manuscript.

† These investigations were carried out with the aid of a grant for research in science made to Washington University by the International Cancer Research Foundation.

1 Loeb, Leo, and Genther, I. T., PROC. SOC. EXP. BIOL. AND MED., 1928, **15**, 809.

appeared communications by Lacassagne[2] and by Harde,[3] which seemed to establish a connection between the differences in the intensity of follicular hormone action during the sexual cycle and the incidence of mammary cancer. Quite recently, however, Bonser[4] in comparing 2 strains of mice differing in the incidence of spontaneous tumors did not find any correspondence between these 2 conditions.

Our own investigations are based on the study of 10 strains of mice markedly differing in their tumor incidence. We considered the following characteristics in the sexual cycle: (1) The duration of the sexual cycles and the number of cycles in individual mice, and the averages in the various strains. (2) The average duration of the periods of keratinization and the relation between the periods of oestrus and dioestrus in the various strains and in the individual mice composing them. (3) The degree of regularity of the oestrous cycles. We may summarize our results by stating that there was no parallelism noticeable between any of these factors and the incidence of mammary cancer either in the individual mice or in the whole strains.

We studied the effect of 2 different diets on the characteristics of the sexual cycle. In a first period of 30 days, the mice were fed solely on a diet of Purina Chow; in a subsequent period of 28 days the diet consisted of a mixture of chow, cracked corn and oats. There was a distinct difference in some characteristics of the sexual cycle during these 2 periods; the regularity in the sexual periodicity was greater in the first set of mice, and in the second series the differences between the nature of the sexual cycles of the different strains were considerably more marked than in the first series, when the pure chow was used. Notwithstanding these differences in the nature of the cycles in these 2 series of experiments, there was a great similarity in certain characteristics of the oestrous cycles in many individual mice and also in the whole strains during both periods. This persistence in the characteristics of the sexual cycle during 2 periods suggests that a factor, perhaps of a genetic nature, inherent in each female mouse is one of the conditions which determines some of the characters of the sexual cycle.

We may then conclude that the great differences in the hereditary tendency to cancer which are found in different strains of mice do not depend upon differences in the characteristics of the sexual

[2] Lacassagne, A., *Compt. Rend. de la Soc. Biol.*, 1934, **115**, 937.

[3] Harde, E., *Compt. Rend. de la Soc. Biol.*, 1934, **116**, 999.

[4] Bonser, S. M., *J. Path. and Bact.*, 1935, **41**, 33.

cycle and in particular not on differences in the intensity of the action of the follicular hormone during the follicular phase of the cycle.

8305 C

## Loss of Blood from Circulation in Various Types of Intestinal Obstruction.

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The theory that the cause of death in intestinal obstruction is a loss of blood from the general circulation into the distended and stagnant vessels of the congested intestinal wall has not in all quarters been entirely discarded. Splanchnic congestion is of course a factor of no importance in obstruction of the duodenum or uppermost jejunum: high obstruction is not therefore considered here, but a series of simple animal experiments has been performed to determine the blood loss from the general circulation in the 3 other varieties of intestinal obstruction which occur clinically—(1) simple low occlusion of the small intestine, (2) closed loop obstruction without strangulation, and (3) closed loop obstruction with strangulation of the veins draining the obstructed loop.

(1) *Simple Low Small Intestine Occlusion.* In each of 9 cats under ether anesthesia a silk ligature was tied tightly around the ileum 18 inches above the ileocaecal junction. Two fine seromuscular knots of silk were placed in the gut wall one foot above, and one foot below the point of obstruction to measure off respectively the lowest foot of obstructed bowel, and an equal adjacent loop of unobstructed bowel. In 2 of the cats the obstructing ligature broke or cut through and the animals recovered. The remaining animals died after periods of 1 to 6 days. In these the 2 measured loops were excised after death and their mucosal surfaces dried by the passage of a cotton pull-through. Only a drop or 2 of blood was lost from each loop during excision from the vessels of the mesentery and of the loop ends. The difference in weight between the measured unobstructed foot of bowel, and the lowest foot of obstructed bowel was taken to represent the maximum possible sur-

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plus of blood in the congested wall of the latter—an obvious overestimate since some part of the increase in weight must be due not to blood but to oedema fluid. This difference varied from 0.23 to 2.5 gm. or expressed as a percentage of the blood volume of the animal (arbitrarily estimated as 7½% of the body weight) from 0.11 to 1% of the blood volume. Since the lowest obstructed loop was in all cases the most congested, this figure multiplied by the number of feet in the small intestine was taken to represent the maximal possible blood loss into the wall of the whole obstructed small intestine. This total loss varied from 0.6 to 5% of the estimated blood volume—a loss comparable with only a trivial external hemorrhage.

(2) *Closed Loop Obstruction without Strangulation.* Seven cats were used for this series. Closed loops of small intestine were prepared by division, and invagination of the divided ends, and were compared with loops of unobstructed bowel of similar length, aboral to them. The lumen was not reconstituted by anastomosis. The closed loops varied in length from one-tenth to one-half of the whole small intestine. The animals died 2 to 6 days after preparation of the loops. In all cases the closed loops were distended and congested. The greatest increase in weight occurred, as would be expected, in the longest closed loop—that which included one-half of the small intestine (a closed loop relatively much longer than those encountered clinically in man) and even here the maximal possible blood loss into the loop amounted to only 11% of the estimated blood volume of the animal. In the shorter loops (one-tenth to one-quarter of the whole small intestine) the maximal possible blood loss was from 1.3 to 3% of the blood volume. In all cases the paleness of the content of the closed loop, and of the fluid in the peritoneal cavity (where any was present) excluded the possibility of measurable loss of blood from the mucous or peritoneal surfaces of the closed loop.

(3) *Closed Loop with Venous Strangulation.* The measurement of loss of fluid from the general circulation was here performed by the rubber bag technique used by Foster and Hausler[1] to exclude strangulated intestine from the peritoneal cavity. A loop of intestine was measured and inserted in a rubber balloon around whose neck a ligature was tied so tightly that the arteries of the mesentery of the strangulated bowel could just be felt pulsating through the balloon wall. After death the weight of the balloon

[1] Foster, W. C., and Hausler, R. W., *Arch. Int. Med.*, 1924, **34**, 697.

and its contents was compared with the weight of the same balloon and a bowel loop of similar length to that strangulated. Here the increase in weight is due solely to the blood lost into the wall of the loop, into the lumen, and outward into the balloon. Twelve cats were used in this series. Two of these, in which leakage at the neck of the balloon gave death from peritonitis, were discarded. In one, where the whole small intestine was strangulated, and the balloon contents compared with intestines of similar length in animals of the same weight, the blood loss was estimated at 45% of the blood volume. In 2 animals, strangulation of one-half of the small intestine gave a blood loss of 43% and 52% of the blood volume. Such a blood loss is in itself sufficient to cause death. In 7 smaller loops, each including one-third of the small intestine, the blood loss into the balloon varied from 22% to 35% of the estimated blood volume —a considerable but not a fatal loss. Using the same technique, Holt[2] found an even greater blood loss in the strangulated intestine of the dog.

*Conclusions.* Blood loss from the general circulation is an unimportant factor in simple occlusion of the intestine, and in closed loop obstruction without strangulation. In venous strangulation in the cat, blood loss is sufficient to cause death if one-half or more of the small intestine be involved. In strangulation even of smaller loops, the blood loss into them, and through them, while not in itself great enough to cause death, is yet sufficient to be a factor of importance. In clinical cases of strangulation, probably some part of the bloodstained transudate is reabsorbed by the peritoneum. No attempt was made in these experiments to estimate or to allow for such reabsorption.

8306 C

## Quantitative Spreading of Fibrinogen in Unimolecular Films.

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Two general methods have been employed to spread proteins on aqueous surfaces in unimolecular films. Gorter and Grendel[1] in-

[2] Holt, R. L., *Brit. J. Surg.*, 1934, **21**, 582.

* University Fellow.

[1] Gorter, E., and Grendel, F., *Proc. Acad. Sci. Amsterdam*, 1926, **29**, 1262.

troduced the method of blowing the protein solution horizontally upon the surface of the water in a Langmuir trough. Hughes and Rideal[2] placed on the water surface solid particles of the protein previously weighed on a microbalance. They could show that the films so obtained, while fluid at the outset, became elastic upon compression (sol-gel transition). Not all proteins, however, are amenable to spreading; examples are fibrinogen[3, 4] and nerve nucleoprotein.[5] These proteins spread very slowly and cannot be induced to form sol, *i. e.*, fluid films. Instead, small amounts float about in elastic patches with equilibrium surface pressures of less than 0.1 dyne/cm., as compared with a pressure of 16 dynes/cm. for gliadin (Hughes and Rideal[2]). The tendency to spread is so slight that because of its greater specific gravity the protein solution usually sinks into the buffer in the trough.

It is highly desirable that methods be worked out for the quantitative spreading of such proteins. In attacking this problem we have worked with fibrinogen because of its ready availability and because methods for its purification are at hand. The fibrinogen was prepared according to the method of Hammarsten as modified by Florkin[6] except that oxalated beef blood was used instead of citrated horse plasma. As recommended by Mellanby[7] all salt solutions were oxalated; by this means the fibrinogen could be reprecipitated 3 times without denaturation. To apply the film we used a 0.2 ml. serological pipette graduated in thousandths. The pipette was sealed to one arm of a stopcock ground for vacuum; the other arm received a small hypodermic needle which was bent so that the protein solution was delivered horizontally with the pipette vertical. Films were spread on McIlvaine buffer, diluted tenfold. All pH determinations were made with the glass electrode.

Confirming the original report of Gorter,[3] fibrinogen films studied under these conditions could not be spread quantitatively. In searching for methods of facilitating the spreading of the protein we tried adding a small amount of alcohol before applying the film. The mixture then spread rapidly, the alcohol quickly evaporating or diffusing into the underlying liquid. Fibrinogen solution containing 10% alcohol showed no precipitate even after 10 hours; with alcohol concentration 20%, precipitation was rapid.

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[2] Hughes, A. H., and Rideal, E. K., *Proc. Roy. Soc. London*, 1932, **A137**, 62.

[3] Gorter, E., *Am. J. Dis. Child.*, 1934, **47**, 945.

[4] Gorter, E., and v. Ormondt, H., *Biochem. J.*, 1935, **29**, 48.

[5] Fourt, L., Bear, R. S., and Schmitt, F. O., *Am. J. Physiol.*, 1935, **113**, 44.

[6] Florkin, M., *J. Biol. Chem.*, 1930, **87**, 629.

[7] Mellanby, J., *Proc. Roy. Soc. London*, 1935, **B117**, 352.

TABLE I.

| Alcohol % | 3.6 | | 7.1 | | 9.5 | |
|---|---|---|---|---|---|---|
| pH | 6.9 | | 2.9 | | 4.9 | |
| | Protein Applied mg. | Area per mg. $m^2$ | Protein Applied mg. | Area per mg. $m^2$ | Protein Applied mg. | Area per mg. $m^2$ |
| | .0096 | .811 | .0096 | .770 | .0258 | .631 |
| | .0120 | .745 | .0144 | .749 | .0379 | .597 |
| | .0168 | .741 | .0192 | .686 | .0439 | .679 |
| | .0192 | .695 | .0264 | .720 | .0540 | .657 |
| | .0240 | .687 | .0336 | .653 | .0568 | .632 |
| | .0278 | .674 | .0420 | .582 | .0572 | .664 |
| | .0342 | .649 | — | — | .0602 | .608 |
| Average Area | | .716 | | .695 | | .638 |
| Film Thickness | | 12.7 Å | | .13.8 Å | | 14.6 Å |

The table illustrates typical results obtained with such films by extrapolation of the linear portion of the surface pressure—area curves to zero pressure. Protein was determined by micro Kjeldahl, using a factor of 6.25. Thickness of film was calculated by assuming the density of fibrinogen to be 1.1. That the fibrinogen was spread quantitatively is shown by the fact that the area per milligram is essentially independent of the amount of protein spread. This is particularly true with the alcohol concentration of 9.5%; with lower concentrations of alcohol, spreading was slower and maximum spreading was prevented by the formation of an elastic restraining ring of protein film, within which subsequent protein emitted from the pipette was held under compression.

The results as tabulated are comparable to those previously reported by Gorter.[4] He has found for most of the proteins examined a maximal spreading of 1.0 $m^2$./mg. at the isoelectric point, and a minimal spreading of 0.1 $m^2$./mg. on either side of the isoelectric point. Our figures are intermediate. The isoelectric point of beef fibrinogen is as yet undetermined, but our preparations showed maximum flocculation in the range of pH 4.2-5.6. According to Hughes and Rideal[2] gelation of gliadin sets in at a thickness of 11-12 Å and an area of about 0.67 $m^2$./mg. This supports the view that the fibrinogen films reported above were always in the gel state, since the area lies between 0.72 and 0.64 $m^2$./mg. and the thickness between 12.7 and 14.6 Å.

# Pacific Coast Section

*Stanford University, October 19, 1935.*

## 8307 C

### Effect of Heat on Viability of Mycobacteria leprae muris.

HAMILTON H. ANDERSON. (With the technical assistance of Russell Leake.)

*From the Pharmacological Laboratory, University of California Medical School, San Francisco.*

Clegg[1] developed a method of cultivating *M. leprae,* using media on which amebae were growing, later heating the cultures to 60°C. for 30 minutes. This procedure destroyed the amebae and symbiotic bacteria and resulted in what Clegg believed to be a pure culture of *M. leprae.* Other workers have stated that on exposing *in vitro* the bacilli of leprosy to 60° for 10 minutes the bacilli are killed.[2] It has been suggested,[3] presumably on the basis of the latter observations, that human leprosy might be susceptible to fever therapy. Denney[4] is attempting to determine if pyrotherapy is of any value in the disease and, if so, whether the effect is a direct thermal one. At his suggestion the following experiment was undertaken.

A leproma was removed from a rat, under sterile conditions, ground with sterile sand and suspended in physiologic (0.9%) saline solution. Seven equivalent amounts were placed in sterile test tubes and exposed to the following temperatures for five hours: 17°, 37°, 39°, 41°, 43°, 50° and 60°C. To serve as test subjects for each respective temperature, 7 groups of 10 rats each were selected of approximately the same weight (average of 200 gm. each) and age, except the first control group (17°C.) which were younger animals, averaging 80 gm. each. One-half cc. of the respective heat-treated saline-suspended *M. leprae muris* was injected subcu-

[1] Clegg, M. T., *Phil. J. Sci.*, 1909, **4**, 77, 403.

[2] Alexandrescu, Marchoux, and Mezinescu. Quoted by Koch, F., *Zentr. f. Haut.-u. Geschlechts-krankh.*, 1932, **40**, 433.

[3] Heiser, Victor, personal communication.

[4] Denney, O. E., personal communication.

taneously into the right lower quadrant of the abdominal wall of each animal.

During the 5 months since the infected material was inoculated the following observations were made: Mycobacteria exposed to 17°C. and 37°C. produced palpable lesions at the injection-site in all animals within 3 months; organisms heated to 39°C. caused lepromata in 7 of 8 surviving rats in 3 months; 60% of the animals given material kept at 41°C. had lesions at the end of 3 months and 75% of the survivors within 4 months. Six of 7 surviving rats injected with the leprous suspension exposed to 43° had palpable leprous masses at the end of 4 months. Temperatures of 50° and 60°C. for 5 hours are sufficient, apparently, to attenuate the *Mycobacteria* and prevent the appearance of lesions, since no animal receiving this material showed signs of leprosy during the observation period of 5 months.

Weight gains were noted only in groups not developing lepromata, *i. e.*, 50°C. (average gain, 27 gm.) and 60°C. (average gain 180 gm.). In the controls given material kept at room temperature, however, an increase due to growth was noted. Intercurrent infection, usually pneumonia, accounted for the death of 26 of the 70 rats originally injected.

*Summary.* Temperatures in excess of 43°C. for 5 hours are required to kill *Mycobacterium leprae muris in vitro,* using rats as a test animal to determine viability over an observation period of 5 months. Fifty degrees C. for 5 hours seems sufficient to attenuate this *Mycobacterium.*

8308 P

## Hemolytic Complement Albumin-Globulin Ratio.

M. C. TERRY. (Introduced by W. H. Manwaring.)

*From the U. S. Veterans Hospital, Palo Alto, California.*

If fresh, cell-free, guinea pig serum in a test tube is repeatedly frozen and thawed without shaking or inverting the tube there will be seen, after a few freezings and thawings, a difference between the upper and lower portions of the tube. If the serum is tinged with hemoglobin the lower portion will be deep red and the upper portion colorless. If there is only a trace of hemoglobin an indica-

tion of what takes place may still be shown by gently tilting the tube back and forth whereupon a transverse movement of heavy oily-looking streaks is seen, somewhere below the middle of the tube, between an amber colored lower portion and a colorless upper portion. If the lower portion is recovered and titered for complement content against sensitized sheep cells this portion will be found to have a higher titer than that of the original whole serum, the difference being greatest if only the extreme lower portion of the concentrated serum be employed.[1]

In one such experiment the contents of the tube, immediately after the last thawing, was divided into 2 equal upper and lower portions and nitrogen determinations were done on the 2 halves and on the original whole serum in addition to complement titrations. The complement titer was higher and the total nitrogen content was greater in the lower half than in the whole serum. The difference in complement value was as 100 to 133; the difference in albumin as 100 to 230; in globulin as 100 to 150. The gain in complement, therefore, was associated with a change in the albumin:globulin ratio, the change consisting in a relative increase in albumin and a relative decrease in globulin. Since the experiment involved no procedure which would seem likely to have any denaturing effect these findings seem significant. The method of freezing and thawing has been employed for concentration of non-colloid solutions and in this experiment with complement the effect of what approximates distilled water in the upper half of the tube must be considered. It may be noted that the effect is the opposite of that produced by the dilution of serum with distilled water, a procedure which is destructive of complement function.[2]

Following the experiments described the investigation was continued by dialysis of guinea pig serum. The globulin precipitate was taken up to volume in normal salt solution and the supernatant albumin fraction was brought to isotonicity by added salt and heated at 55° for 15 minutes. Additions of these fractions were made to fresh serum which was then titered as complement. It was found that albumin addition raised the titer slightly while globulin addition lowered it considerably and in larger quantity extinguished it completely. Whether albumin and globulin from other sources would similarly affect guinea pig complement is a matter now under investigation.

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[1] Plant, Arthur S., *Br. Med. J.*, 1933, **2**, 414.

[2] Sachs u. Teruuchi, *Berl. kl. Woch.*, 1907, 16, 17, 19.

Guinea pig serum was now kept before an electric fan until the volume was reduced to about 1/10 by "pervaporation".[3] The gross change was obviously the removal of water. The complement titer of the serum thus highly concentrated was only about doubled.

Since pervaporation presumably made no change in the relation of the various protein fractions to each other and resulted in a relatively slight increase in complement titer while freezing and thawing caused a demonstrable change in those relations and was accompanied by a notable increase in titer it would appear that the increase in titer in the latter case was due to the changed relation of protein fractions, the demonstrated change being a relative increase of albumin and a relative decrease of globulin.

8309 P

## Vacuolization During the Water Exchanges of Cells.

JAMES L. LEITCH. (Introduced by S. C. Brooks.)

*From the Department of Zoology, University of California, Berkeley, and Department of Marine Biology, Carnegie Institution of Washington, Dry Tortugas, Fla.*

In a study of the water exchanges of the eggs of the sea urchin, *Echinometra lucunter,* the appearance of vacuoles was noted during one phase of the swelling process. When single eggs of this species were observed while swelling in 60% sea water, 2 different equilibria were found during each of which the measurements of the egg diameters remained constant for a period of from 20 to 30 minutes. The first of these occurred after approximately 60 minutes' exposure to the experimental solution and the second after 120 minutes, the eggs now exhibiting a somewhat smaller volume. The shrinkage occurring between these 2 equilibria was accompanied by active vacuolization, the vacuoles appearing in the central portion of the egg and migrating to the cortical layer. Although the emptying of these vacuoles to the outside was not seen, it was inferred from the fact that no accumulation of vacuoles could be detected at the periphery even though additional vacuoles were continually migrating in that direction.

Just[1] raised the question whether the consideration of an egg as

[3] Farber, Lionel, *Science,* 1935, **82**, 158.

[1] Just, E. E., *Protoplasma,* 1930, **10**, 24.

a simple osmotic system as proposed by Lucké and McCutcheon[2] should not be modified in the light of the wide occurrence of vacuolization in the eggs of *Arbacia punctulata.* Tests of the capacity of the eggs of *Echinometra lucunter* to be fertilized and to develop when returned to normal sea water at different times during the swelling process showed that, after the end of the first equilibrium, the eggs no longer react normally. This would indicate that vacuolization only occurs in these eggs after they have been injured and that therefore the normal, uninjured eggs may be considered as simple osmotic systems.

No detailed study of this phase has yet been completed on the eggs of Echinometra so that at this time one cannot explain the mechanism behind this vacuolization. However, Heilbrunn,[3] from observations on vacuolization in the eggs of *Arbacia punctulata,* concluded that this phenomenon is an internal surface precipitation reaction. This is solely an explanation of the mechanism forming the vacuoles and does not explain the factors which are operating to initiate and limit the vacuolization.

A detailed report of these experiments together with non-solvent volume determinations on the eggs of *Echinometra lucunter* will be published in the Papers from the Department of Marine Biology of the Carnegie Institution of Washington.

## 8310 P

### Nucleotide Nitrogen Content of Certain Tissues of the Dog and Rabbit.

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Recent investigations have attributed several properties to the nucleotides which occur in the tissues. The most important of these properties is the participation of adenosine triphosphate in the phosphorylation of the hexose arising from the hydrolysis of glycogen. This is a very necessary procedure in the anaerobic formation of the lactic acid of the muscle.[1] However, as is well known, all tissues

[2] Lucké, B., and McCutcheon, M., *Physiol. Rev.*, 1932, **12**, 68.

[3] Heilbrunn, L. V., *Protoplasma Monographien,* 1928, **1**, Chap. XIV.

[1] Lohmann, K., *Biochem. Z.*, 1931, **241**, 50.

do not show the same behavior in their ability to form lactic acid.[2] As a method of approach to the study of this difference and its possible regulation by the nucleotide level of the tissue, a study of the quantitative distribution of nucleotide nitrogen among the various tissues has been carried out.

TABLE I.

| Tissue | Nucleotide Nitrogen per 100 gm. Tissue | | | |
|---|---|---|---|---|
| | Dog | | Rabbit | |
| | Arithmetical Mean | Extreme Variation | Arithmetical Mean | Extreme Variation |
| | mg. | mg. | mg. | mg. |
| Whole Blood | 3.4 | 3.2- 3.9 | 6.6 | 6.4- 6.7 |
| Whole Brain | 13.3 | 12.5-15.5 | 19.3 | 17.7-20.7 |
| Intestine | 13.3 | 8.7-18.1 | — | — |
| Pancreas | 24.5 | 19.2-27.7 | — | — |
| Kidney | 21.8 | 17.6-25.5 | 30.9 | 28.0-33.6 |
| Spleen | 22.6 | 21.3-24.5 | — | — |
| Liver | 27.2 | 25.8-28.3 | 47.8 | 46.2-49.0 |
| Whole Heart | 28.9 | 26.0-31.0 | 35.0 | 32.0-37.9 |
| Muscle | 50.8 | 46.5-56.9 | 60.9 | 54.1-64.4 |

Table I contains the results of the analyses of certain tissues of the dog and rabbit for their nucleotide nitrogen content. The quantitative method of Kerr and Blish[3] was used. All results were obtained from duplicate check samples. A minimum of 5 animals was used for each tissue determination.

## 8311 C

## Fibrinolytic Staphylococci.*

R. R. MADISON. (Introduced by W. H. Manwaring.)

*From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

Aoi[1] found that 88% of all Staphylococcus strains isolated from "pusturating foci" are capable of dissolving Congo-red-fibrin. Ap-

[2] Warburg, O., *Biochem. Z.*, 1927, **184**, 484.

[3] Kerr, S. E., and Blish, M. E., *J. Biol. Chem.*, 1932, **98**, 193.

* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of the Stanford Medical School.

[1] Aoi, F., *Kitasato Arch. Exp. Med.*, 1932, **9**, 171.

plying their routine plasma-clot technic, however, Tillett and Garner[2] found only an "occasional" strain that was thrombolytic. Moreover, these few strains were of very low fibrinolytic titer, requiring approximately 24 hours to cause demonstrable softening of the plasma-clot.

We have tried to harmonize the 2 sets of data by titrating 145 local strains of *S. aureus* or *albus*† for fibrinolysin, using the more delicate isolated-fibrin technic.[2] Data thus obtained are summarized in Table I.

TABLE I.

Fibrinolytic Function of Staphylococci.

Serial dilutions of 24-hour broth cultures of each of the 145 strains were added to the routine serum-free human fibrinogen-thrombin complex, volumes, dilutions, temperatures, etc., being identical with those used by Tillett and Garner.[2] The highest serial dilution liquefying the resulting fibrin-clot by the end of 2 hours, was assumed to contain one lytic unit. From this, the approximate number of lytic units per cc. was calculated.

Cultures giving no lysis by this technic were re-titrated by the 10-fold enzyme-concentration method,[3] this giving the approximate number of decifibrinolytic units per cc.

The table is based on the average of 3 titrations. Each strain is assigned to the nearest recorded serial dilution, strains giving titers from 4 to 7, for example, being recorded under titer 5.

| *Staphylococcus aureus* or *albus* isolated from: | No. and % of lytic and non-lytic strains in each group — Fibrinolytic units per cc. of broth culture | | | | | | | Total No. of strain in each group |
|---|---|---|---|---|---|---|---|---|
| | 10 | 5 | 2.5 | 1 | 0.05 | 0.1 | 0 | |
| Group A | 3 | 8 | 5 | 8 | 1 | 2 | 3 | 30 |
| Internal human tissues | *37%* | | *43%* | | *10%* | | *10%* | *100%* |
| Group B | 1 | 4 | — | 3 | 4 | 6 | 60 | 78 |
| Superficial human tissues | *6%* | | *4%* | | *13%* | | *77%* | *100%* |
| Group C | — | — | — | — | — | — | 24 | 24 |
| Veterinary tissues | *0%* | | *0%* | | *0%* | | *100%* | *100%* |

The table shows that 77% of all local strains of Staphylococcus isolated from superficial human lesions (acne, boils, nasal sinus, etc.) are without demonstrable fibrinolytic function, even when tested by the 10-fold enzyme-concentration method. In contrast, 90% of all local strains isolated from internal human lesions (septicemia, osteomyelitis, empyemia, cellulitis, etc.) are fibrinolytic, 37% of them yielding as many as 5 to 10 lytic units per cc. of broth culture.

None of the 24 local veterinary strains (horse, cow, dog, swine) is capable of liquifying human fibrin.

[2] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

† The strains and clinical histories used in this survey were kindly furnished by the various hospitals, clinics, diagnostic laboratories and veterinary institutions of the San Francisco Bay Region.

[3] Madison, R. R., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 445.

The fibrinolytic factor formed or secreted by the Staphylococcus is not of the same immunologic specificity as the streptofibrinolysin. Streptococcus antiserum‡ containing as many as 1000 antifibrinolytic units per cc. does not neutralize the staphylofibrinolysin.

## 8312 C

### Survival of Two Depancreatized Dogs Treated with Insulin.*

I. L. CHAIKOFF.

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Soon after the discovery of insulin, attempts were made to maintain completely depancreatized dogs with insulin. Macleod[1, 2, 3] found that dogs receiving insulin and a diet of meat and sucrose survived for 8 months, whereas the addition of raw pancreas to their diets permitted the survival of 2 completely depancreatized dogs for about 4 years. Although the diets employed were deficient, Macleod concluded that raw pancreas was essential for the survival of the depancreatized dogs for periods longer than 8 months. More recently Hershey[4] reported that lecithin was of value in this connection. Hershey and Soskin[5] state that the ingestion of lecithin supplements enables depancreatized dogs to live indefinitely and that it cures the hepatic insufficiency that appears from 6 weeks to 11 months after pancreatectomy. However, the longest period of survival reported by these workers was 1 year and 3 months. Later, Best and Hershey[6] and Best, Ferguson and Hershey[7] found that the

‡ The antistreptococcus serums used in these and other tests were kindly furnished by: Eli Lilly and Co.; The Cutter Laboratory; Parke, Davis and Co.; Lederle Laboratories; and E. R. Squibb and Sons.

* The expense of this investigation was defrayed in part by a grant from the Research Board of the University of California, Berkeley. The insulin was generously donated by Eli Lilly and Company.

1 Allen, F. N., Bowie, D. J., Macleod, J. J. R., and Robinson, W. L., *Brit. J. Exp. Path.*, 1924, **5**, 75.

2 Macleod, J. J. R., Carbohydrate metabolism and insulin. Longmans, Green and Co., Ltd., New York, 1926.

3 Macleod, J. J. R., *Lancet*, 1930, **219**, 383.

4 Hershey, J. M., *Am. J. Physiol.*, 1930, **93**, 657.

5 Hershey, J. M., and Soskin, S., *Am. J. Physiol.*, 1931, **98**, 74.

6 Best, C. H., and Hershey, J. M., *J. Physiol.*, 1932, **75**, 49.

7 Best, C. H., Ferguson, G. C., and Hershey, J. M., *J. Physiol.*, 1933, **79**, 94.

essential factor in lecithin was choline. It should be noted that the longest period of survival of a depancreatized dog reported in the latter publications was somewhat more than 2.5 years.

The present report deals *only* with the period of survival of the depancreatized dog. Following complete pancreatectomy, 2 dogs were injected twice daily with insulin and fed a diet containing the nutritional requirements known to be essential for the normal dog. They received twice daily a mixture consisting of lean meat, sucrose and bone ash in amounts previously specified.[8] In addition, vitamin supplements were added twice a week, A and D in the form of cod liver oil, B as a concentrate obtained from rice polishings. On such treatment 2 depancreatized dogs have survived for well over 4 years and at present are alive. A description of these dogs follows:

*Dog DA*—female; completely depancreatized March 11, 1931.

*Dog DC*—female; born November 20, 1930; completely depancreatized September 1, 1931.

Both dogs are at present (October, 1935) in good nutritional condition. The cataractous formations in the lenses† of these dogs, as well as the state of the blood lipids,[8] have been noted elsewhere. The postabsorptive blood sugars of these animals have always been above normal, hypoglycemia having never been observed in either of these dogs during their entire stay in the laboratory.

The relation of choline to the survival of dogs DA and DC remains to be considered. It is known that the largest part of the choline present in lean meat is contained in lecithin,[9] and that the choline present in other forms is a negligible amount.[10] Both meat and cod liver oil‡ have been used by Best *et al.*[5, 7] in the diets that produced fatty livers in depancreatized dogs. It is the prevention of fatty livers which Best *et al.* claim is effected by feeding choline and which they regard as essential for the survival of such dogs for long periods. The vitamin B concentrate§ employed by us is obtained from an aqueous extract of rice polishings and is fed in such small amounts that, regardless of the amount present, in all probability it can furnish only negligible amounts of choline, far less

[8] Chaikoff, I. L., and Kaplan, A., *J. Biol. Chem.*, 1934, **106**, 267.

† The cataracts found in dogs DA and DC have been described in the PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 237, where these 2 animals are recorded as dogs 7 and 2 respectively.

[9] Bloor, W. R., *J. Biol. Chem.*, 1927, **72**, 327.

[10] Alles, G. A., *Physiol. Rev.*, 1934, **14**, 276.

‡ According to phospholipid analysis of the cod liver oil used in this study, dogs DA and DC received negligible amounts of choline by this means.

§ 100 gm. of this extract contained less than 15 mg. of phospholipid.

than that already contained in the meat. If, as Best *et al.* claim, choline reduces the fat content of the livers of depancreatized dogs to normal levels, then the fact that the diet used in the present investigation produced livers containing as high as 43% fatty acids,[11] a value higher than that hitherto reported, is further evidence that choline could have played no part in the survival of dogs DA and DC for 4.5 and 4 years respectively.

The fact that in the present study one dog was kept alive for 4.5 years and another for 4 years lends doubtful support to the view that raw pancreas, lecithin supplements or choline supplements are essential for *maintenance of the depancreatized dog.* Indeed, dogs DA and DC, which have never received raw pancreas, lecithin supplements or choline supplements during their entire stay in this laboratory, have lived longer than depancreatized dogs reported to have received either one or other of these 3 constituents. It is obvious, therefore, that a minimum period of 4.5 years must elapse in the survival of a depancreatized dog before it can be claimed that a given substance is essential in keeping it alive. A period shorter than this falls within the time in which a depancreatized dog can live without ingesting either raw pancreas or lecithin supplements or choline supplements.

It is now well known that depancreatized dogs maintained under the dietary conditions of the present investigation are not in all respects normal. The eyes of both DA and DC show extensive bilateral cataractous formations.[12] It was found in this laboratory that lenticular opacifications may appear as early as one year after pancreatectomy. The blood lipids are markedly disturbed, a reduction in all lipid constituents, and in particular cholesterol esters, occurring soon after pancreatectomy.[8] The livers of these animals show enormous accumulations of lipids,[11] a change that may make its appearance as early as 10 weeks after pancreatectomy despite the insulin treatment. Nevertheless 2 facts must be noted about depancreatized dogs:

1. Survival for as long as 4.5 years is possible in the presence of these changes in liver, blood and lenses, provided such a dog is maintained on a diet containing meat, sucrose, bone ash and the necessary vitamins.

2. Judging by weight and subcutaneous fat, dogs DA and DC are in good nutritional state despite the pathological changes in the

[11] Kaplan, A., and Chaikoff, I. L., *J. Biol. Chem.*, 1935, **108**, 201.

[12] Chaikoff, I. L., and Lachman, G. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 237.

3 tissues listed above. Except for the first week or two following pancreatectomy, during which time their appetites were somewhat poor, both dogs have had voracious appetites throughout their stay in the laboratory. Both dogs are still alive and cannot be distinguished from normal dogs in appearance or reactions.

## 8313 C

### Measurement of Reagin in Non-Syphilitic Sera.*

CHARLES W. BARNETT, RICHARD B. JONES AND GEORGE V. KULCHAR.
(Introduced by M. L. Tainter.)

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The reporting of serological tests for syphilis as positive or negative implies a qualitative difference between the bloods of syphilitic and non-syphilitic individuals. This concept assumes the presence in the serum of syphilitic patients of a substance designated as "reagin", which is lacking in patients without syphilis. In syphilitic patients, treated or untreated, it is highly probable that small amounts of reagin persist even though the serum is negative to the usual tests. As yet there is no evidence that reagin may not also be present in non-syphilitic sera.

If reagin is to be demonstrated in non-syphilitic sera, it is necessary to use a test so sensitive as to be invariably positive, to concentrate the serum, or to increase the reagin content to a point where it can be detected by the usual tests. We have chosen the last method, adapted from the work of Schreus and Foerster[1] who studied the Wassermann reaction in syphilitic patients treated to seronegativity. By adding to the sera of these patients subthreshold amounts of positive sera, these workers were able to obtain positive reactions. In place of the Wassermann we have substituted the Kline test because of its simplicity.

Suspensions are prepared from standard Kline antigen in the usual way. A stock reagin solution is prepared from positive serum after inactivation for 15 minutes at 56°C. Double precipitation is

* This work has been aided by a grant from the Rockefeller Fluid Research Fund.

[1] Schreus, H., and Foerster, R., *Z. f. Immunitäforsch. u. Exp. Therap.*, 1934, **82**, 53.

carried out in half saturated ammonium sulphate as described by Schreus and Foerster. The second precipitate is dissolved in a minimal amount of normal saline. The reagin content of this stock reagin solution decreases less rapidly than that of untreated serum according to these workers. This solution is now diluted 10, 20, 30, 40, and 50 times with saline and the Kline test[2] is performed with these dilutions to determine the approximate titre. Further tests are done on dilutions made in unit steps within the range indicated by the first titration and the exact titre is thus obtained.

Reagin is added to the serum to be titrated as indicated in Table I.

TABLE I.*

| Test No. | Serum cc. | Reagin cc. | Saline cc. |
|---|---|---|---|
| 1 | .05 | — | — |
| 2 | .04 | .001 | .009 |
| 3 | .04 | .002 | .008 |
| 4 | .04 | .003 | .007 |
| 5 | .04 | .004 | .006 |
| 6 | .04 | .005 | .005 |
| 7 | .04 | .006 | .004 |
| 8 | .04 | .007 | .003 |
| 9 | .04 | .008 | .002 |
| 10 | .04 | .009 | .001 |
| 11 | .04 | .01 | — |
| 12 | .039 | .011 | — |

*These quantities are too small for accurate measurement; the solutions in each test are mixed in the same proportions but in larger quantities and .05 cc. of this mixture is taken for the test.

The quantities indicated in Table I are selected to bring the total volume of fluid in each test to .05 cc. as required in the Kline test. When serum is replaced by normal saline, all tests up to No. 11 should be negative, and 11 and 12 positive. This result will be obtained if a dilution of stock reagen to 1/5 of its titre is used. Thus if the stock reagin has a titre of 1:36, the dilution to be used is 1:36/5 or 1:7.2. Such a dilution is prepared and the series of tests shown in Table I is set up using saline instead of serum to verify the original titration. The tests are then repeated with serum inactivated for 15 minutes at 56°C. If no reagin is present in the serum, there will be no shift in the end point and test No. 11 will again be the first to be positive. If, however, reagin is present, this point will be shifted and the degree of this shift will indicate the amount of reagin present.

There is no standard unit of reagin. For the purpose of this study we have taken as our unit of reagin the least amount that can

[2] Kline, B. S., and Young, A. M., *J. Lab. and Clin. Med.*, 1927, **12**, 477.

be detected by this technic. The end point is the first test showing agglutination. The reading of this end point is made possible by having a series of negatives on one side and a series of increasing positives on the other for comparison. Sharp end points are obtained only when freshly prepared cholesterin solutions are used in preparing the Kline antigen suspension. All tests have been read independently by the 3 of us and the errors in the readings have never been more than one-tenth of a unit.

If such a series of tests is done with an unknown serum we may find that the first positive test is No. 7 instead of No. 11 as in the saline control series. This indicates the presence in the serum of 0.4 units of reagin. Since the serum has been diluted by the addition of saline and reagin, this figure must be multiplied by 5/4 to obtain the actual reagin content. This would give us in this example $0.4 \times 5/4 = 0.5$ units.

In order that our unit of reagin will be constant the sensitivity of the test must remain fixed. The fixed sensitivity of the test was verified by the preparation of 9 separate pairs of antigen suspensions. Five of these pairs gave identical results when used on the same serum or reagin solution, and 4 disagreed by only 0.1 units. Fresh suspensions and suspensions 24 hours old showed the same agreement. Our unit of reagin is apparently constant.

In titrating the same serum but using different solutions of reagin, identical values for reagin content were obtained. When syphilitic serum was substituted for reagin solution, the readings were likewise unchanged.

The reagin content of the sera of 42 apparently normal individuals was determined. In this small group no significant variation of the reagin titre with sex or age was observed. The results are summarized in Table II.

TABLE II.

| No. of Sera | Units of Reagin |
|---|---|
| 27 | 1.0 |
| 12 | 0.9 |
| 2 | 0.8 |
| 1 | 0.6 |

The sera of 3 patients with nephrosis and one with myasthenia gravis, all without evidence of syphilis, gave values within the same range. The blood of 6 patients with treated syphilis and with negative Wassermann reactions were also titrated. The results are given in Table III.

TABLE III.

| | Units of Reagin |
|---|---|
| Seronegative primary, treated 6 mo. | 0.9 |
| " " " 6 " | 1.9 |
| Seropositive " " 2 yr. | 1.0 |
| Secondary, treated 1 yr. | 1.5 |
| Asymptomatic neurosyphilis, treated 2 yr. | 1.0 |
| Taboparesis | 1.5 |

When the serum contained more than one unit of reagin, the determinations were made by the method described for the titration of stock reagin solution.

The results of reagin determinations on various dilutions of the same serum are shown in Table IV.

TABLE IV.

| | Reagin Content (Units) | |
|---|---|---|
| Dilution | Determined | Calculated |
| Undiluted | 1.0 | 1.0 |
| % | | |
| 25 | 0.75 | 0.75 |
| 30 | 0.6 | 0.5 |
| 75 | 0.35 | 0.25 |

The titrated and calculated values agree within 0.1 unit in each dilution. This indicates that the method is accurate over a wide range.

Reagin is evidently present in the sera of non-syphilitic individuals. There is no proof that this reagin is identical with that found in syphilitic sera but it reacts in the same way to the Kline precipitation test. The difference between syphilitic and non-syphilitic sera seems to be a quantitative rather than a qualitative one. Measurements of reagin by the method described here are accurate to about 0.1 unit.

The determination of the reagin content of the serum in the various phases of syphilis and in other diseases, particularly those in which false positives have been reported, is contemplated.

The method described here should provide a direct measure of the sensitivity of serological tests. With a sensitive test, such as the Kline, very small amounts of reagin added to normal serum will give a positive reaction. With an insensitive test, larger amounts would probably be necessary. The amount required should provide an index of sensitivity.

*Summary.* 1. A method is described for the detection and quantitative measurements of reagin in non-syphilitic sera. 2. Non-

syphilitic sera are regularly found to contain small quantities of reagin.

8314 P

## Fenestration of Nuclei of Lymphocytes: A New Diagnostic Sign in Infectious Mononucleosis.

EDWIN E. OSGOOD.

*From the Department of Medicine, University of Oregon Medical School, Portland.*

The abnormality of the nuclei of some of the lymphocytes in infectious mononucleosis described below was first observed in 1933. Since then I have found it in all of the 12 cases of infectious mononucleosis studied and it has not been observed in any other condition, although differential counts are made on about 50 to 100 bloods a day in the laboratory under my direction and the technicians were asked to look especially for these fenestrations. In 2 or 3 instances fenestrated nuclei were observed before the diagnosis of infectious mononucleosis had been made by other methods, and this diagnosis was later confirmed by finding the typical large lymphocytes, a positive Paul and Bunnell test, and by the clinical course.

The fenestrated nuclei* appear at first glance to be nuclei containing multiple nucleoli, but careful inspection shows that in the Wright's stained smear there are actually multiple holes, piercing the nucleus in various directions. In those which are parallel to the light beam through the microscope it is obvious that they are holes; but the majority will, from the laws of chance, not have this direction and their diagonal course through the nucleus gives the appearance of an oval-shaped area of decreased density which may or may not have a clear patch at one end. These may be differentiated from nucleoli by the facts that they are too numerous and that their background is the same color as the rest of the nucleus but paler, while in nucleoli the color is a pure blue with no tinge of red in it.

These fenestrations may be present in the nuclei of either the normal or large lymphocytes but are most often seen in the smallest of the otherwise normal lymphocytes. They are usually present in a relatively small proportion of the lymphocytes and may not be

* Slides and colored illustrations showing these morphologic characteristics were shown in the scientific exhibit at the 85th annual session of the American Medical Association in Cleveland, June, 1934.

observed if only 100 cells are examined in making the differential count. However, by thorough search, I have been able to find them in slides from all cases of infectious mononucleosis which I have studied.

The nature and cause of these fenestrations remain to be determined.

8315 C

## Phagocytosis of Malaria Parasites by the Neutrophil Leukocytes of the Marrow.*

EDWIN E. OSGOOD.

*From the Department of Medicine, University of Oregon Medical School, Portland.*

It has long been known that malaria plasmodia or their disintegration products must be phagocytized by the neutrophil cells, because pigment granules are frequently seen in the neutrophils in smears from the blood. However, I have never seen, and have been unable to find a record of anyone else who has seen, intact or only partially digested malaria parasites within neutrophil cells in the blood.

The development of the sternal puncture technic for obtaining human bone marrow during life[1] made possible the study of marrow smears in malaria. Examination of these smears revealed not only a higher percentage of the red cells invaded but the presence in the neutrophil leukocytes of malaria parasites in all stages of digestion, from the intact organism to the residual collection of pigment

* Slides and illustrations showing this phenomenon were demonstrated in the scientific exhibit at the 85th annual session of the American Medical Association in Cleveland, June, 1934.

[1] Young, R. H., and Osgood, E. E., *Arch. Int. Med.*, 1935, **55**, 186.

granules. Most of the phagocytizing leukocytes were staff cells but phagocytosis was observed also in segmented neutrophils and neutrophil metamyelocytes. Examination of blood smears made at about the same time that the marrow was taken revealed no evidences of phagocytosis of malaria parasites except the occasional presence of pigment granules in the neutrophils.

Such phagocytosis has been found in all sternal marrows from the 5 cases of inoculation tertian malaria which have so far been available for study. It seems reasonable to believe that this phagocytosis will be found to occur in naturally occurring tertian malaria and in the other forms of malaria.

8316 P

## Structure of Colored Compound Formed in the Sullivan Reaction for Guanidine.

M. X. SULLIVAN AND W. C. HESS.

*From the Chemo-Medical Research Laboratory, Georgetown University, Washington, D. C.*

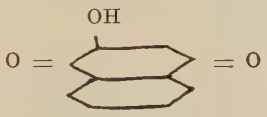
Sullivan[1] found that when guanidine is heated with 1.2 naphthoquinone-4-sodium sulphonate and alkali a brown colored solution is formed which on acidification with concentrated HCl and concentrated $HNO_3$ gives a striking red complex while all other compounds tested, amino acids, amines, etc., go to yellow. Even amino guanidine and methyl guanidine are yellow. The formation of the red compound in the presence of HCl and $HNO_3$ was utilized as a test with a high degree of specificity for free guanidine.

In order to determine the structure of the colored compound formed, one gram of guanidine hydrochloride was dissolved in 10 cc. of water and to this was added 4 gm. of the naphthoquinone in 25 cc. of water, with a little of the quinone still in suspension. The well stirred mixture was made definitely alkaline with 10 cc. of 5N NaOH and the solution was brought to boiling on a water bath, then quickly cooled to 10-15°C. and acidified with 15 cc. of concentrated HCl followed by 15 cc. of concentrated $HNO_3$. The heavy red precipitate was centrifuged and washed free of acid by water. It was then dried in a desiccator and in an oven at 80°C. Without any attempt to obtain maximum yields the weight of the

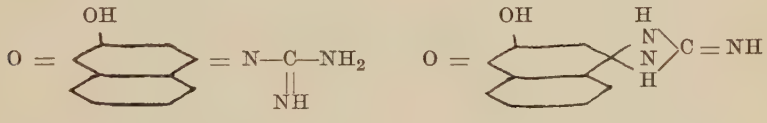
[1] Sullivan, M. X., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 106.

material purified for analysis was 1.8 gm. or 80% of the theoretical yield. The compound was little soluble in cold water, slightly soluble in alcohol and rather insoluble in acetone and ether. It dissolves in dilute NaOH with a strong red color. The M.P. on quick heating was 242-245°C. with decomposition. The nitrogen content was 19.35%. The same decomposition point obtained whether the precipitant was concentrated HCl, concentrated $HNO_3$, or mixtures of these, and mixed melting points of these precipitates showed no change.

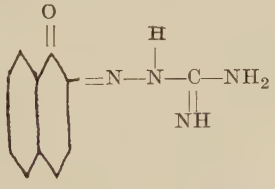
On acid hydrolysis, 20% HCl or better alcohol $H_2SO_4$, 10% by weight of concentrated $H_2SO_4$ in 95% alcohol, the products obtained without attempt at optimum hydrolysis and fractionation of products were hydroxynaphthoquinone



M.P. 187-88°C. in 74% yield of the theoretical and guanidine in 42% yield. To give these products the beta naphthoquinone guanidine complex must be: (A) or (B)



Analogous anilidonaphthoquinones were early shown by Zincke[2] and Liebermann[3] to yield hydroxynaphthoquinone on hydrolysis while as shown by Thiele and Barlow[4] compounds of the type



would yield alpha naphthol on hydrolysis. We have made the guanidine complex corresponding to Thiele and Barlow's compound. Its melting point is 265-267°C. with decomposition. Its nitrogen content is 21.19% and on acid hydrolysis it yielded more

2 Zincke, Th., *Ber. Chem. Ges.*, 1881, **14**, 1493.

3 Liebermann, C., *Ber. Chem. Ges.*, 1881, **14**, 1664.

4 Thiele, J., and Barlow, W., *Ann. der Chem.*, 1898, **302**, 311.

or less alpha naphthol. The reaction product of 1.2 naphthoquinone-4-sodium sulphonate and guanidine as made in the Sullivan guanidine reaction, with a nitrogen content of 19.35%, a decomposition point of 242-245°C. and yielding hydroxynaphthoquinone on hydrolysis is explainable on the basis of formula (A) or (B).

8317 C

## The Effect of Hypophysectomy upon Mammary Gland Development and Function in the Guinea Pig.*

WARREN O. NELSON.

*From the Department of Anatomy, Yale University.*

Studies on mammary gland development and lactation during the past few years have indicated that the ovarian hormones are responsible for the proliferation of the glands while the lactogenic hormone of the anterior pituitary is concerned with the initiation and maintenance of milk secretion. However, the possibility of a direct action of the anterior pituitary, as at least a contributing factor in mammary development, cannot be disregarded and obviously requires investigations on the character of development in hypophysectomized animals. Asdell and Seidenstein[1] have reported that hypophysectomized rabbits treated with oestrone and progesterone show mammary development comparable to that obtained in the intact animal. In addition to the question of a direct influence of the hypophysis on mammary growth there are uncertainties in regard to the necessity of the hypophysis for the initiation and maintenance of lactation.[2]

Hypophysectomy has been carried out in the guinea pig by a parapharyngeal approach. The operation is well tolerated, operative and early post-operative mortality being low, but deaths, probably due to hypoglycemic crises, are frequent during the first week. This has been successfully combatted to some extent by the routine administration of glucose during the first 10 days after operation.

* Aided by a grant from the National Research Council, Committee for Research in Problems of Sex. Grant administered by Dr. Edgar Allen. Also aided by a grant from the Anna Fuller Foundation.

[1] Asdell, S. A., and Seidenstein, H. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 931.

[2] Nelson, W. O., *Endocrinology*, 1935, **19**, 187.

Animals have been maintained for as long as 51 days after hypophysectomy.

Of a group of hypophysectomized male guinea pigs which were subsequently treated with oestrone† (40 R.U. daily) 6 survived for at least 5 weeks. The character of mammary development as judged by the nipple size, gland spreads, and histological section was of a degree comparable to that observed in normal animals.[3] Autopsy showed pituitary fragments in 2 of these animals.

Six male guinea pigs which had carried functional ovarian grafts for 7 to 10 weeks, and 3 males and 4 females which had been injected with 40 R.U. oestrone daily for 5 to 7 weeks were hypophysectomized. At the same time the ovarian grafts were removed, or in the case of the oestrone-injected animals treatment was suspended. Such animals, with intact hypophyses, will invariably lactate within 60 hours.[2] Nine of the above animals survived more than three days. Five animals failed to lactate during a period of 5 to 10 days, while 2 showed a scanty and 2 about the normally expected degree of lactation. Autopsy revealed complete hypophysectomies in all animals which had failed to lactate and in one of those which had presented a scanty secretion. In the remaining animals pituitary fragments were found.

Three pregnant guinea pigs were operated within a few days of term. One animal died before parturition, but the remaining 2 delivered successfully. One failed to lactate and the other showed only a scanty serous secretion which did not persist. Hypophysectomy was complete in both animals.

Three females which had passed through normal pregnancies were operated 7 days after delivery. Lactation is well established at that time and normally will continue for about 2 weeks. Lactation declined the day after operation in one animal, but she died before observations could be completed. However, the other 2 survived, showing a rapid decrease in secretion after the second day. On the fifth day milk was no longer present in the glands.

In these studies it has been observed that under suitable stimulation the guinea pig mammary glands undergo development in the absence of the pituitary. This development appears to be entirely comparable to that seen in the intact animal. However, lactation does not occur in suitably prepared animals when the hypophysis is absent. This evidence supports the idea[2] that the initiation of lacta-

† The oestrone used in this study was theelin-in-oil and was kindly supplied by Dr. Oliver Kamm of Parke, Davis & Co.

[3] Nelson, W. O., and Smelser, G. K., *Am. J. Physiol.*, 1933, **103**, 373.

tion depends upon activity of the anterior hypophysis following the removal of the inhibitory influences exerted by the estrogenic hormone. Furthermore, it was observed that the maintenance of lactation depends upon the presence of the anterior hypophysis.

8318 P

## Differentiation and Function of Heterotopic Autoplastic Transplants of the Amphibian Hypophysis.

WAYNE J. ATWELL.

*From the Department of Anatomy, the University of Buffalo.*

Conflicting evidence has been obtained regarding the ability of the epithelial hypophysis to differentiate and function in heterotopic transplants in amphibia. Blount,[1] using *Amblystoma punctatum,* failed to obtain differentiation of the epithelial hypophysis independent of the infundibulum. Etkin[2] made successful single and multiple transplants in *Rana sylvatica* with a minimum of brain tissue, but he made no attempt to remove all possible adherent brain.

The writer, in 1931, removed the hypophysis from 18 specimens of *R. sylvatica* at the tail-bud stage and transplanted it in the same individual to a location between the right otic vesicle and the hind brain. Care was taken not to include any brain tissue or any entoderm. Eight of these animals gave evidence of function of the hypophysis according to one or more of the criteria enumerated below, although 5 were sacrificed at a stage before evidence of anterior lobe function can be obtained. In the present year similar operations were attempted on 75 *R. sylvatica,* 30 *R. pipiens* and 50 *A. punctatum.* From the experiments of these 2 years serial sections of head, thyroid and gonad-adrenal regions have been studied from 64, 8 and 5 animals of the above species, respectively. In addition, the condition of the hypophysis and the thyroid has been determined at autopsy in 10 *A. punctatum,* while 30 animals of this species are still alive. For control study records and sections from more than 200 normal, or completely or partially hypophysectomized amphibia, mostly *R. sylvatica,* were available.

The pigmentary condition of the animal was taken as an indicator

[1] Blount, R. F., *Proc. Nat. Acad. Sc.,* 1930, **16**, 218; *J. Exp. Zool.,* 1932, **63**, 113; 1935, **70**, 131; *Anat. Rec.,* 1935, **61**, Suppl., 6.

[2] Etkin, W., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1653.

of the state of function of the pars intermedia.[3] Function of the anterior lobe proper was evidenced by (1) definite progress towards metamorphosis, (2) preservation of the normal size and structure of the thyroid, (3) maintenance of the size and lipoid content of the adrenal cortex.

For a transplant to be deemed entirely successful, study of an animal was required to show: (1) entire absence of epithelial hypophysis from the orthotopic position, (2) characteristic hypophyseal tissue in the region of the transplant, (3) evidence of function of the transplant as given above. Twenty-six such specimens of the wood frog and 2 of the leopard frog, but none of the salamander, have been found in the material studied. A few of the living animals show early signs of metamorphosis but the condition of the hypophysis in these is of course unknown.

All of the "successful" cases exhibited evidences of function of the anterior lobe, except 3 which were sacrificed prior to complete differentiation of the thyroid and the appearance of legs. These 3 and 11 others showed a condition of normal or partial darkness indicative of pars intermedia function. In 1 of these 11 animals the transplant apparently had been made directly into the brain wall. In 5 others there was definite contact, even intermingling, of the transplant with the acoustic ganglion. In the remaining 5 contact with tissue of neural origin was questionable.

The transplant usually has been found inside the cranium between the internal ear and the hind brain; sometimes it was imbedded in the cartilage of the cranial floor, and sometimes it was entirely outside of the cranium. In only the single case mentioned above was there contact between the transplant and the brain. In most instances it is easy to identify the anterior lobe by its histological structure, but identification of pars intermedia tissue has been possible in only a few cases.

It is concluded that transplantation of the primordium of the epithelial hypophysis, independent of brain or fore-gut, may be followed by differentiation and function of the transplant in *R. sylvatica* and *R. pipiens*. Similar experiments with *A. punctatum* so far have been unsuccessful. Differentiation and function of the anterior lobe proper take place independently of contact with neural tissue, and are independent of the pars intermedia. There is some evidence, not yet entirely conclusive, that contact with neural tissue is necessary for differentiation and function of the pars intermedia.

[3] Atwell, W. J., *Science*, 1919, **49**, 48; *Anat. Rec.*, 1934, **58**, Suppl., 48; Atwell, W. J., and Holley, E., in press.

It seems certain, however, that this tissue need not be that of the infundibular region.

8319 P

## Immunization of Rabbits with Inactive Vaccinia Virus.

I. J. KLIGLER AND H. BERNKOPF.

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It is generally accepted that immunity to virus diseases can only be produced by an infection with a live, even though highly attenuated, virus. From time to time experimental evidence contrary to this view has been published, but the results have not been convincing. In this preliminary report references may be limited to experiments with vaccine virus. Gordon[2] used virus heated to 57°C. for 30 minutes, a period insufficient to kill this virus. Hunt and Falk[3] reported positive results with virus treated with a weak solution of formalin, but Olitsky and Long[6] showed that vesicular stomatitis virus thus treated still contained live virus. The most careful work has been carried out by Bland,[1] who tested his vaccine for live virus and used both heat and formalin killed virus. He reported positive results in guinea pigs and equivocal results in rabbits.

The results obtained by Bland, as well as our own observation[4, 5] on the antigenic nature of purified phage and of typhus rickettsia in cultures, suggested that the failure to induce immunity with dead virus was due to the relatively small amount of antigen contained in tissue suspensions of viruses. In the phage work it was found that an amount of suspension containing not less than 20 million plaques was necessary to produce an antiserum with moderate neutralizing power. In the case of rickettsia it was estimated that an infected guinea pig brain weighing 3 gm. would have a maximum only of 12,000,000 organisms, and this seemed sufficient reason why infected lice or cultures made an efficient vaccine, whereas, a whole guinea pig brain produced at best only a slight degree of immunity.

[1] Bland, J. O. W., *J. Hyg.*, 1932, **32**, 55.

[2] Gordon, M. H., *Med. Counc. Rep.*, 1925, No. 98.

[3] Hunt, L. W., and Falk, I. S., *J. Immunol.*, 1927, **14**, 347.

[4] Kligler, I. J., and Olitsky, L., *Brit. J. Exp. Path.*, 1931, **12**, 172.

[5] Kligler, I. J. and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

[6] Olitsky, P. K., and Long, P. H., *J. Exp. Med.*, 1928, **47**, 835.

The experiments reported below were undertaken in order to test the validity of this view in regard to vaccine virus.

The vaccine used consisted of a 10% suspension of infected testicle, or an eluted virus of the same strength, either heated 2 hours at 56°C., or treated with formalin (final concentration 1.0%), and kept 24 hours in the incubator and one or more weeks in the icebox. The vaccine was tested for live virus by intracutaneous injections of 1.0 cc. of the stock material into susceptible animals.

The virulence of the virus suspension and the eluate were titrated on a rabbit skin before heating. The strength ranged between $10^{-6}$ and $10^{-7}$. In other words 1.0 cc. of the 10% suspension contained about 1,000,000 infective doses. On the basis of the phage work about 20 cc. would be required to produce a measurable degree of immunity. The neutralizing power of the serum of the rabbits to be vaccinated was titrated before immunization. Immunity after the treatment was tested by intradermal injections of different dilutions of an active virus as well as by titration of the neutralizing power of the serum.

Animals given 1 or 2 injections of 5 cc. of the vaccine gave entirely negative results. Those, however, receiving 3 or more injections gave results which indicated that an immunity had developed. Below is given several protocols of experiments on rabbits receiving 3 or more injections:

*Rabbits* 446 *and* 553 received 4 injections of 5 cc. of a 10% testicular suspension—446 formolized, 533 heated. Titre of untreated virus suspension 1:10,000,000. Sera (1:5) mixed with virus dilutions 1:10,000 to 1:1,000,000 gave no neutralization. Injections given 4 cc. i.p. and 1 cc. i.c., at 3-day intervals. Blood taken 7 days after last injection.

*Rabbit* 533—serum 1:5 neutralized (1 hr. at room temperature and 24 hr. in icebox) 1:1,000,000 and 1:100,000 but not 1:10,000 dilution of virus. Intracutaneous test: 1:10,000±, 1:100,000—. Control: 1:10,000,000+. *Rabbit* 446—serum 1:5 neutralized 1:1,000,000 and 1:100,000 only partially, delayed reaction; intracutaneous test: all dilutions positive, reactions delayed.

*Rabbits* 489, 456 received 3 injections eluted virus; each 4 cc. i.p. and 1.0 cc. i.c.; 489 received formolized, 456 heated material. Titre of eluate: positive 1:1,000,000. Titre of serum before immunization: serum 1:5, virus 1:10,000 to 1:1,000,000, no neutralization. Blood taken 7 days after last injection.

*Rabbit* 489—skin test 1:10,000 to 1:1,000,000. Next day all points showed equally extensive, infiltrated, inflamed areas. Control still negative. Two days later infiltration more extensive, control

positive with normal progression of lesions. A definite allergic reaction. Neutralization: serum 1:5, virus 1:10,000 to 1:1,000,000; after 3 days 1:10,000+, 1:100,000 and 1:1,000,000—, controls on same rabbit positive at all points; after 6 days all points positive, reaction delayed; slight neutralization.

*Rabbit* 456—skin test not carried out because animal died. Neutralization test with serum same as 489.

*Rabbit* 301—heated eluate, 7 injections; each time 5 cc. i.p. and 0.2 cc. i.c. at intervals of 2 days. Eluate positive in dilution of 1:1,000,000. Neutralization test: whole serum 1:10 and virus 1:10,000 negative. Blood taken 6 and 10 days after last injection. Neutralization test: whole serum and 1:10 with virus 1:10,000; on second day control positive, test negative; on fifth day control ++++, test only slight infiltration ±. Skin test: virus 1:10,000 negative. This animal had definite skin immunity and its serum considerable neutralizing power.

## 8320 C

## Effect of Sodium Fluoride upon Experimental Thyroid Poisoning.

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Several authors[1–4] have reported beneficial effects from the oral and intravenous exhibition of the fluorides in the treatment of hyperthyroidism, rationalizing the procedure, in some instances at least, upon the well-known enzyme inhibiting action of this halogen. Laboratory findings offer a scientific basis for this treatment, since moderate doses of the fluorides produce *(a)* a diminished tissue respiration[5] and anaerobic glycolysis[6] in excised organs, *(b)* a decreased oxygen consumption and lactic acid production in muscle,[7] and *(c)* a sharp decrease in the oxygen consumption[2] and the carbon dioxide[8] production in the intact animal. Goldemberg[2] states that

[1] Woakes, E., *Lancet*, 1881, **1**, 497.
[2] Goldemberg, L., *Semana Medica*, 1932, **39**, 1659.
[3] Gorlitzer, V., *Med. Klin.*, 1932, **28**, 717.
[4] Reveno, W. S., *J. Michigan State Med. Soc.*, 1934, **33**, 359.
[5] Phillips, P. H., and Stare, F. J., *J. Biol. Chem.*, 1934, **104**, 351.
[6] Dickens, F., and Simer, F., *Biochem. J.*, 1929, **23**, 936.
[7] Lipmann, F., *Biochem. Z.*, 1928, **196**, 3; 1929, **206**, 171.
[8] Gorlitzer, V., *Arch. f. exp. Path. u. Pharm.*, 1932, **165**, 443.

thyroxine is precipitated *in vitro* by fluorides in alkaline medium. He attributes the beneficial effects of the drug in toxic goiter to an *in vivo* inactivation of thyroxine and an inhibition of oxidases and other tissue ferments.

If this depression of metabolism results from an inactivation of thyroxine and its precursors in the body, it seems logical to postulate that the administration of a fluoride should protect an experimental animal from the toxic action of orally administered desiccated thyroid glands.

TABLE I.

The Effect of Sodium Fluoride upon Thyroid-Fed Rabbits.

| Drug | Dosage | No. Animals | Aver. Survival Died | Period |
|---|---|---|---|---|
| | mg./Kg. | | | days |
| Untreated Controls | | 16 | 0 | |
| Sodium Fluoride | 2.5 | 12 | 0 | |
| | 5 | 11 | 0 | |
| | 10 | 3 | 0 | |
| Desiccated Thyroid | 100 | 11 | 9* | 41 |
| Sodium Fluoride<br>Desiccated Thyroid | 5<br>100 | 12 | 9* | 31 |
| Sodium Fluoride<br>Desiccated Thyroid | 10<br>100 | 9 | 6* | 41 |

*Survivors killed for histologic examination.

The accompanying data (Table I), obtained from the daily intravenous administration of 1% sodium fluoride to 21 adult rabbits which were also poisoned with 100 mg. per kg. per diem of desiccated thyroid glands, given orally in capsule, show no significant variation in the rapidity of weight loss or the life span of the fluoride treated animals from the thyroid controls. Histologic examination of the thyroid glands of both groups likewise showed that fluoride did not prevent the characteristic flattening of the acinal cells produced by thyroid feeding. The quantity of sodium fluoride used (5 to 10 mg. per kg.) was slightly larger than the 1 to 4 mg. per kg. dosage recommended by the clinician. To determine whether these dosages would induce fluorine cachexia in the rabbit, and whether the route of administration of the drug would influence its toxicity, the weight curves of the fluoride controls, groups of which received the drug either orally (stomach tube), intramuscularly, or

intravenously, were compared with untreated controls over the 5-month experimental period. No significant change was found in the weight curves following 2.5 to 5 mg. orally, whereas intravenous administration of these doses actually induced a more rapid weight increase than in the controls. Intramuscular administration retarded weight gain, probably as a result of local tissue injury. Toward the latter part of the experiment, those animals on 10 mg. per kg. intravenously failed to gain weight in the normal fashion. These results would indicate intravenous administration of daily doses of less than 5 mg. per kg. to be relatively free from toxicity for the adult animal.

These findings do not lend support to the hypothesis of an *in vivo* thyroxine inactivation and a concurrent inhibition of the ferments concerned in metabolism, providing a constant supply of thyroxine is available by thyroid feeding. Neither do they necessarily condemn the therapeutic use of fluorides nor preclude the possibility that fluorides may prevent the elaboration of excessive thyroxine, or its precursors, by the hyperactive gland of thyrotoxicosis, either by a specific inhibitory action on the glandular cells or by a diminution of the iodine content or interference with the iodine utilization of the gland, as recently postulated by Reveno.[4]

## 8321 C

## Normal Curve of Leucocyte Count of the Albino Rat Over a 24-Hour Period.

DOUGLAS WARNER. (Introduced by Dr. Francis Gilchrist.)

*In cooperation with the Department of Zoology, Pomona College, Claremont, Calif.*

Those who have reported on the rhythm of leucocyte count in man are not in complete agreement. The following work pertains to the blood of man: Reinke,[1] Galambos,[2] Mauriac and Cabouat,[3] Stetson,[4] and Medlar,[5] agree that there are variations within the day of the leucocyte count which can be considered normal. Japha,[6]

[1] Reinke, J., *Beitr. z. path. Anat. u. z. Allg. Path.*, 1889, **5**, 439.

[2] Galambos, A., *Folia Haemotologica*, 1912, **33**, 153.

[3] Mauriac, P., and Cabouat, P., *Paris Med.*, 1921, **39**, 407.

[4] Stetson, R. P., *Arch. Int. Med.*, 1927, **40**, 488.

[5] Medlar, E. M., *Am. J. Med. Sci.*, 1929, **177**, 72.

[6] Japha, A., *Jahrb. f. Kinderhlk*, 1900, **52**, 242.

Turk,[7] Fletcher and Mitchel,[8] Shaw,[9] Smith and McDowell,[10] and Martin[11] find that the count is normally higher in the afternoon than in the morning. Doan and Zerfas[12] and Sabin, Cunningham, Doan and Kindwall[13] report short period rhythms as well as the longer cycle. The latter report an hourly rhythm for the total count and a morning to afternoon tide with a peak in the afternoon. They find that this tide is due to an increase in the neutrophiles which have also the hourly rhythm. The lymphocytes, they report, have a 15-minute cycle and are relatively constant as to tide. These workers conclude that the possible normal variation for a given individual is covered within the day. Shaw[9] reports 2 tides a day. Each tide is of 12 hours' duration. The forenoon tide reaches a peak in the afternoon and ebbs in the evening. The night tide, starting in the evening, reaches a peak after midnight and fades in the morning. Medlar[5] finds no consistent hourly rhythm but does concede that each individual may have a rhythm of his own. He states that the total normal variation of any individual is covered within an hour.

Goldberg and Lipskaia[14] state that mental and physical labor bring about an increase in the neutrophiles at the expense of the lymphocytes. Shaw[9] and Sabin[13] and her coworkers both find the neutrophiles responsible for the tides.

In the experiments herewith reported, conditions have been devised so as to standardize the internal factors and to make uniform the external factors which might affect the cell count.

Standardization of internal factors: 1. Food and water were taken from the animal at least 2 hours before the experiment. 2. The animals in the stock room were kept on an unvarying diet. 3. Males only were used. 4. All animals were Wistar rats from the strain known as the Experimental Colony strain. 5. Each rat was of a different litter.

Uniformity of external factors: 1. The animals had been isolated from females since 10 days of age. 2. The experimental ani-

[7] Turk, W., *Dtsch. med. Wchnschr.*, 1912, **38**, 2186.

[8] Fletcher, E. G., and Mitchel, A. G., *Am. J. Dis. Child.*, 1927, **34**, 807.

[9] Shaw, A. F. B., *J. Path. and Bact.*, 1927, **29**, 389.

[10] Smith, C., and McDowell, A. M., *Arch. Int. Med.*, 1929, **43**, 68.

[11] Martin, H. E., *J. Physiol.*, 1932, **75**, 113.

[12] Doan, C. A., and Zerfas, L. C., *J. Exp. Med.*, 1926, **46**, 511.

[13] Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A., *Johns Hopkins Hosp. Bull.*, 1925, **37**, 14.

[14] Goldberg and Lipskaia, from Piney, A., 1928, Recent Advances in Haematology. P. Blakiston's Son & Co., Inc., 1928, 2nd ed.

mals were placed in small individual cages 12 hours before the experiment was to begin. 3. Fairly constant temperature was maintained for 12 hours before and for the duration of the experiment. 4. Light was kept at a constant value. 5. Handling and the resultant excitation were kept at a minimum.

In all, 53 animals, with an age spread of from 60 to 550 days, were used.

All cell types common in the circulating blood of man were found. The white blood cells have been considered as divisible into 3 main groups which may in turn be subdivided. These groups and their subdivisions follow:

I. Lymphocytes. This cell varies in size from 6½μ to 15μ with a definite mode at 9.4μ. 200 cells were measured and plotted. A typical bell curve resulted, rather than a 2-mode curve as the literature would lead one to expect. The nucleus is well defined, being lumpy in appearance as contrasted with the monocytes. The nuclear material stains a dark, slightly reddish purple, and makes up all but about 1/6th of the total diameter of the cell as seen in 2 dimensions. The cytoplasm stains a faint, dull blue. Granules are occasionally present in small numbers in the cytoplasm. The lymphocyte is the predominant cell in the blood of the rat, averaging 53.24% of the total count. Occasionally there was observed a sharply defined nucleolus stained very densely and shaped either as a washer or round.

II. A. Monocytes. This cell varies in size from 11μ to 17μ with the average at 13μ. The cytoplasm is practically colorless and usually irregular in shape. The nucleus accounts for only about ½ of the total diameter of the cell. The nucleus stains more lightly than that of the lymphocyte and the color is bluer. The structure appears to be stringy, rather than lumpy. Frequently, the cell seems to have been fixed during a period of streaming. This appearance is apparent in both the nucleus and the cytoplasm. Their number accounts for 2.74% of the total count.

B. Transitional. This cell varies in size from 13μ to 17μ. It resembles the monocyte with the exception that the nuclear material is clearly split at least one-half the way through. A few transitionals were found with lymphocyte-type nuclei. Frequency of appearance is 0.39%.

III. A. Polymorphonuclear neutrophilic leucocyte. This cell varies in size from 9⅓μ to 17μ with a mode at 11¼μ and with an average of 12 1/5μ. The nucleus is always "ropy" in texture and tends to be less lobulated and of more uniform diameter than in the

human cell. The cytoplasm is usually colorless with a few granules. They are purple in color, taking both the blue and the red stain. Although there is considerable variation in the relative number of lymphocytes and polymorphonuclear cells during different parts of the day, the average frequency of the latter is 39.18%.

B. Polymorphonuclear basophilic leucocyte. The few cells of this type measured varied from 10μ to 13μ with an average size of 11½μ. The nuclear material is more homogeneous and less ropy than that of the neutrophile. The cytoplasm contains many very large granules which stain definitely blue. The average frequency of this cell is 0.13%.

C. Eosinophiles. The average size of this cell is 12.6μ with a spread of from 9⅓μ to 17μ. The nuclear material resembles that of the basophile as compared with the neutrophile. The nucleus as seen 2-dimensionally occurs in one of 3 shapes:

1. As a perfect circle in 58% of the cases.
2. As a figure eight in 29% of the cases observed.
3. With a second twisting, making 3 loops in 11% of the cases.

The cytoplasm is of a definite light red color. Although magnification of 1425× was used, only occasionally could the individual granules be made out because of their smallness. Some animals were found with a large number of eosinophiles. This was taken to indicate the presence of intestinal parasites. Eliminating these animals, the average frequency was 2.20%.

D. Metacytes. The average size of this cell was found to be 11¾μ with a variation from 7μ to 17μ. The characteristically notched nucleus is made up of material exactly resembling the lymphocyte nucleus. The cytoplasm is usually slightly more abundant and tends to stain less densely. Its frequency of appearance was found to be 1.93%.

E. Stabkernige of "Staff" cell. The average size of the few cells measured was 11¾μ with a spread from 10⅓μ to 14μ. The description of this cell would be the same as that for man. Its frequency of appearance is 0.19%.

There were also observed a few eosinophilic metacytes and eosinophilic "Staff" cells. Only 2 myelocytes were positively identified.

At 2-hour intervals, blood was taken from the animal from a single slash in the tail. A total white count was made. Two chambers were counted and an average struck. At the same time, smears were prepared. These were stained with Wright's within 4 hours and mounted under balsam as soon as dry. Counts were taken for at least 14 hours. In most cases they were continued for 20 or more

hours. One-fourth of the animals were started at 6 A. M.; one-fourth at noon; one-fourth at 18 o'clock (6 P. M.); and one-fourth at 24 o'clock (12 midnight). It was found in most cases that the first 2 or 3 readings showed a drop, regardless of what time of day the animals were started. It was also noted that after 18 hours unexpected variations occurred. For these reasons, counts taken in the intervening period were made the basis of the study. To restate, by staggering the starting time of different individuals, it has been possible to eliminate consistently, the first 3 and the last few readings from each animal and then, by recording all readings remaining for each animal, show a composite curve over a 24-hour period.

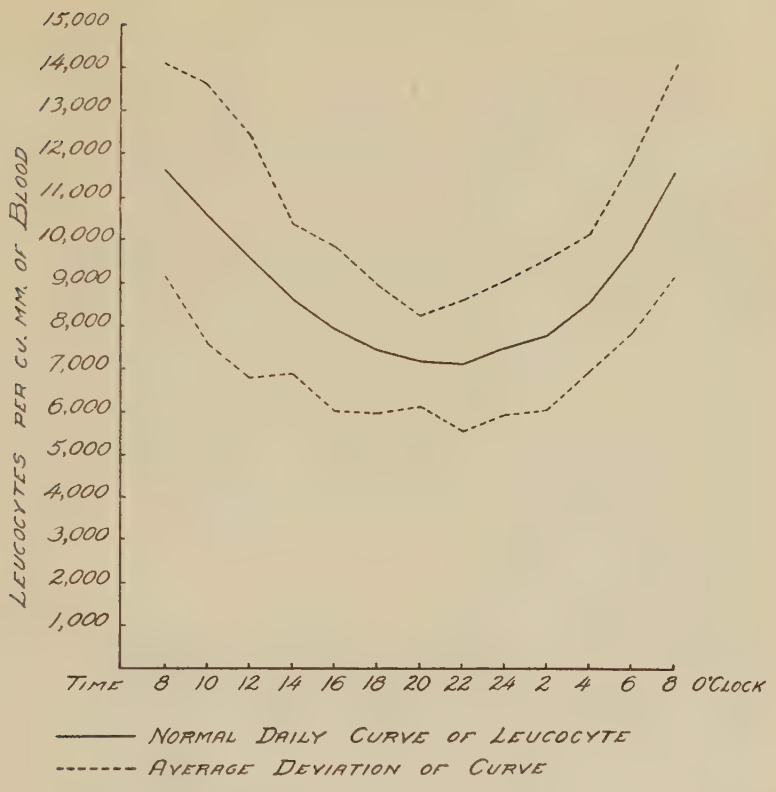
The consistent level of the red counts taken experimentally indicates that change of blood volume or compression of blood has not played any part in the white cell variation.

By this method there has been found, for total count of white cells, a definite daily tide. The low point is at approximately 22 o'clock. A gradually increasing rise (logarithmic type curve) reaches its peak at 8 o'clock and rather suddenly falls away in a straight line until about 14 o'clock when it starts to level out, returning to the low point again at 22 o'clock. The average deviation shows an overlap as plotted against the curve.

When the 3 major cell types are plotted in a cumulative graph, it is seen that all 3 follow roughly the same curve, although the majority of the change is due to the lymphocytes. The polymorphonuclear group reaches its low point at 18 o'clock, 6 hours before the lymphocytes. When the actual percentage values of polymorphonuclear neutrophilic cells are divided into the difference in percentage between that count and the subsequent count, and all these values for a given 2-hour period taken together and averaged, there results a figure which represents the rate of change. Plotting these figures reveals that this type showed a remarkably strong and persistent gain from 16 until 24 o'clock. When the lymphocytes are considered in the same manner, there is found little of significance other than the expected reciprocal drop from 16 until 24 and 8 until 16. The metacytes taken alone show their greatest increase in absolute number for a period ending 6 hours prior to the sudden rate change increase of the polymorphonuclear neutrophilic leucocytes.

Thirty-one consecutive counts were taken at 15-minute intervals in an attempt to confirm the work of Sabin, *et al.*, reporting short rhythms of the different cell types. The results were negative.

The following conclusions are indicated: 1. A diurnal tide, with




its low point at 22 o'clock (10 P. M.) ascending at an increasing rate till shortly after 8 o'clock (A. M.) and then falling at a decreasing rate until the low point is again reached, has been experimentally demonstrated for the white rat. 2. Lymphocytes are more responsible for this tide than the polymorphonuclear group, although all cell types share in the loss and gain. 3. The total normal variation of the white cell count of a rat occurs each day. 4. The lowest level is approximately 62% of the highest level. 5. Eight types of white blood cells found in the circulating blood of the normal white rat have been briefly described. 6. The frequency of appearance of these cells has been given as based on the study of 164 differential counts.

Acknowledgment: To Dr. C. D. Leake, School of Medicine, University of California, for greatly needed criticism and advice. To Drs. Hilton and Gilchrist, Pomona College, for their help throughout. To the SERA funds as administered by Pomona College for two student helpers, Misses Whiteside and Hough, who assisted with differential counts.

8322 C

# The Chylomicron Count in Diabetes Mellitus.

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Gage[1] has called attention to the fact that following a fatty meal the blood is loaded with minute fat droplets which persist for a time and then disappear. Ludlum, Taft and Nugent[2] have made some studies on these particles and think that they are neutral fat droplets, stabilized by blood proteins. They think, therefore, that the counting of these particles gives a fair index of the fat content of blood. Bloor[3] has shown that these chylomicrons are increased in a diabetic dog. Several other clinical conditions have also been reported. It was thought that it would be interesting to examine blood of fasting diabetic patients and make counts, comparing these with normal fasting blood.

The chylomicrons were counted by Gage, and Ludlum, Taft and Nugent by noting the number of particles in a given area viewed through the micrometer eyepiece ruling while the slide was illuminated with a dark field condenser. In attempting to carry out this count, using a Zeiss cardioid condensor with an arc light, difficulties were encountered which are not mentioned by Gage, or Ludlum, Taft and Nugent.

Under brilliant illumination there were, indeed, the bright moving particles as described by these authors. These particles were not all of the same size. Some particles were about 0.1 micron in size and exhibited a slow Brownian movement. In addition to these, however, there were swarms of particles which graded slowly into invisibility. The most minute of these exhibited very rapid Brownian movements. It was as impossible to count these as to count gnats in a swarm. The number of these smaller particles depends greatly upon the intensity of illumination. Clearly, the count of such a mixture of large and small particles is a very inaccurate thing. In addition to this difficulty, it was noticed that if the plasma was allowed to stand for some time the number of particles increased. Under these circumstances the values given can only be considered as estimations.

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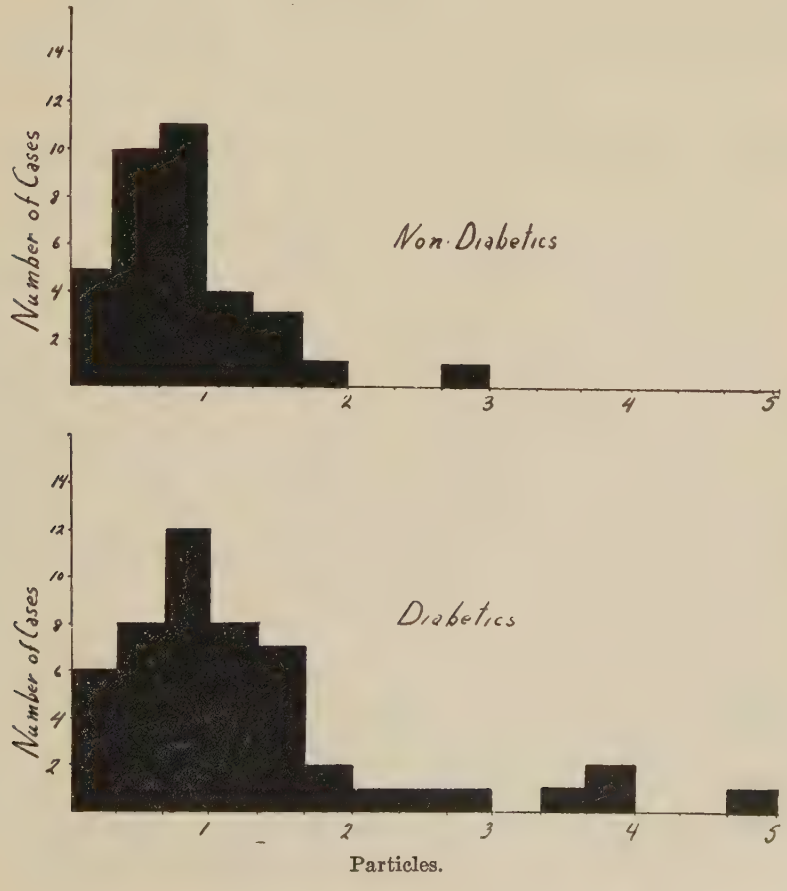
[1] Gage, S. H., and Fish, P. A., *The Cornell Veterinarian*, 1921, **11**, 143.

[2] Ludlum, S. D., Taft, A. E., and Nugent, R. L., *Colloid Symposium Monographs*, 1931, **8**, 269.

[3] Bloor, W. R., *J. Biol. Chem.*, 1916, **26**, 417.

The oxalated plasma was obtained from fasting specimens of blood. The chylomicrons were counted by placing a drop of plasma upon a thin slide and covering with cover slip. The oil lens was lowered until no dancing particles were seen and the position noted by reading the collar of the fine adjustment. The lens was slowly raised and the number of bright particles which flashed into view within an area of the eyepiece ruling was counted. This was continued until no more particles were visible in motion. The depth of the fluid was then read off from the collar. It was thus possible to calculate the number of particles per volume. In some cases, it was necessary to dilute the plasma with distilled water until the particles were far enough apart to be counted.

The distribution of particles in 46 non-diabetic and 56 diabetic cases is shown. The majority of cases in both groups fall about the same mean. Among the diabetics there are cases with very



high counts. In addition to those shown in the chart there were 3 diabetic cases showing 10 particles per 100 cubic micra and one showing 45 particles. When the clinical records of the patients who showed very high counts were examined, it was found that the samples of blood were taken before their diabetes was well regulated.

We can conclude, therefore, that controlled diabetics have a fasting chylomicron count about the same as that of normal persons. The unregulated diabetics have a higher count than normal.

In the majority of cases cholesterol determinations were done on the blood. There was no correlation with the chylomicron count, very high counts being found in some instances with low normal cholesterol values, and low counts being obtained in cases with moderately high cholesterol values. The determinations of total lipid have not been made on the material.

8323 P

## Effect of Pituitrin Injection in Rabbits on Serum Osmotic Pressure and Blood Picture.

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Dodds and Noble[1] reported that a single massive subcutaneous dose of pituitrin in rabbits resulted in a severe anemia on the 4th to 5th day after injection. This anemia was macrocytic, hyperchromic and accompanied by a marked leucocytosis, reticulocytosis, and an increase in bile. Dodds and Noble are inclined to entertain the possibility "that the control of blood destruction by the reticulo-endothelial system may be vested outside the system and may reside in the posterior lobe of the pituitary gland."

In view, however, of the well-known antidiuretic action of pituitrin, and the high water content of a rabbit's normal diet, it was thought that this anemia might well be due to serum osmotic changes resulting from water retention, rather than to a "hemoclastic principle" of the posterior pituitary.

In over 20 preliminary experiments on 15 healthy adult rabbits, pituitrin was injected subcutaneously in the dose reported by Dodds,

[1] Dodds, E. C., and Noble, R. L., *Nature*, 1935, **135**, 788.

200 units/kg. of body weight (10 cc. pituitrin* per kilo). The animals were kept in individual metabolism cages on a diet of cabbage, carrots and lettuce *ad libitum*. At frequent intervals the serum osmotic pressure values were determined by the vapor pressure method of Hill,[2] and serum specific gravity alterations were followed by the falling drop method of Barbour and Hamilton.[3] Complete blood counts were frequently taken, and the reticulocyte, hematocrit, fragility to hypotonic saline solutions, icteric index and mean cell diameter values were closely followed. Urines were tested for hemoglobin and urobilinogen, and stools for occult blood. Body weight, urine volumes and specific gravities were recorded daily.

The essential data of our experiments have been briefly summarized as average figures in Table I.

TABLE I.
Average Urine and Blood Changes* in Rabbits Following Subcutaneous Pituitrin.

| | Normals | Post-injection value | % change | Max. change noted on: |
|---|---|---|---|---|
| | | | | day |
| Urine volume, 24 hr. | 500 cc. | 35 cc. | —93 | 3 |
| Urine specific gravity | 1.011 | 1.029 | +164 | 3 |
| Serum osmotic pressure† | 0.866% | 0.642% | —26 | 5 |
| Serum specific gravity | 1.0221 | 1.0178 | —20 | 4 |
| Red blood corpuscles | 5.39 mil. | 3.19 mil. | —40 | 7 |
| Hemoglobin | 12.0 gm. | 8.0 gm. | —33 | 8 |
| Hematocrit | 34.0% | 20.0% | —40 | 7 |
| Fragility | | | | |
| Hemolysis: Begins | 0.50% NaCl | 0.42% NaCl | 16 | — |
| Hemolysis: Complete | 0.40% NaCl | 0.27% NaCl | 33 | — |

*Figures representing the maximal changes observed in each experiment are averaged in this table.

†Expressed as percentage concentration of an isosmolar NaCl solution.

It is clear from this table that accompanying the evident anemia, there is also a marked decrease in urine output, a definite blood dilution, an abnormally low level of osmotically active substances in the serum and interesting alterations in the *in vitro* fragility of the red corpuscles.

Body weight was variable, representing a balance between gain due to water retention and loss resulting from poor appetite which often followed the injection of pituitrin. As a rule the icteric index

*This pituitrin, CE1138-D, was kindly furnished by Dr. Oliver Kamm of the Parke, Davis & Company.

2 Hill, A. V., *Proc. Roy. Soc.*, 1930, **127**-A, 9.

3 Barbour, H. G., and Hamilton, W. F., *J. Biol. Chem.*, 1926, **69**, 625.

remained well under the value for clinical jaundice. The reticulocyte increase was not marked unless the anemia was severe.

The most marked anemia so far encountered is depicted in Table II.

TABLE II.

Adult rabbit; ♂; pre-injection weight: 1.92 Kg.; Pituitrin subcutaneously, 200 units per Kg.

| | Pre-Inject. Value | Max. Change to: | 3 Weeks after Inject. |
|---|---|---|---|
| Body weight | 1.92 Kg. | 1.52 kg. | 1.66 Kg. |
| Urine volume, 24 hr. spec. | >500 cc. | 40 cc. | >500 |
| Urine specific gravity | 1.010 | 1.020 | 1.010 |
| Serum osmotic pressure* | 0.880% | 0.647% | 0.868% |
| Serum specific gravity | 1.0218 | 1.0172 | 1.0206 |
| Red blood corpuscles | 6.25 mil. | 1.38 mil. | 3.16 mil. |
| Hemoglobin | 13.5 gm.—% | 4.5 gm.—% | 7.5 gm.—% |
| Hematocrit | 34.5% | 11.0% | 25.0% |
| Fragility | | | |
| Hemolysis begins | 0.52% NaCl | 0.46% NaCl | — |
| Hemolysis complete | 0.36% NaCl | 0.28% NaCl | — |
| Reticulocytes | 0.3% | 49.7% | 6.1% |
| Icterus index | 6 | 10 | 6 |

*See note bottom Table I.

There is a definite parallelism between the decrease in serum osmotic pressure and the RBC count. Inasmuch as the specific gravity change observed, which is consequent upon the dilution of plasma proteins, corresponds in order of magnitude to that observed for the osmotic pressure change, the mechanism of osmotic dilution seemingly must result from water retention rather than electrolyte excretion. Furthermore, this plasma dilution due to water retention is far insufficient in itself to account for the marked reduction in cell count. At present we are inclined to interpret the anemia as a hemolytic one due to hypotonic plasma. That the plasma can become a decidedly abnormal environment for red cells after pituitrin administration is shown by the fact that in one experiment the serum osmotic pressure was reduced to such a striking extent as to be isosmolar with a NaCl solution of 0.561%.

The recovery from the anemia occurs gradually and parallels increased urine output and the slow return of serum osmotic pressure to normal.

These experiments are still in progress as well as others designed to determine (1) the minimal and optimal pituitrin dosage, (2) the quantitative relationship between the *in vitro* fragility values and the level of plasma hypotonicity at which *intravascular* hemolysis occurs and (3) the prevention of the anemia by maintenance of a normal osmotic environment in the blood.

8324 P

# Unmodifiability of Locomotor Coordination in Amphibia, Demonstrated by the Reverse Functioning of Mutually Exchanged Right and Left Limbs.

PAUL WEISS.

*From the Department of Zoology, University of Chicago.*

According to the so-called resonance principle,[1] a spinal limb center produces motor impulses specific for each individual muscle, to which the motor nerves, specified appropriately by their muscles, respond selectively. A reflex pattern is an orderly timed (coordinated) sequence of such specific impulses. Left and right half of a spinal center act separately.[2] Corresponding left and right muscles are of identical specificity.[3]

A supernumerary muscle, through its specified nerve, picks up the messages emitted for the normal muscle of the same name in the district; therefore, a supernumerary limb grafted into the district of a normal limb acts always simultaneously and identically with the latter. If the supernumerary and the nearby normal limb are of opposite laterality (*e. g.,* a right leg grafted near a left leg), their movements are mirror images of each other, and if the supernumerary limb can reach the ground, its movements necessarily counteract the effects of the normal limb.[4] The animals never "learn" to remedy this impediment, at least not in the cases where the normal limb remains functional.

There the question arose as to whether, if the normal limbs were removed and limbs of opposite laterality were grafted in their places, the function of the latter would still be reversed as it is in the presence of the normal limbs, or whether some adjustment in the emission pattern of the reflex might not make the grafts serviceable to the body.

In a number of salamanders (*Amblystoma punctatum* larv.), fully developed right and left fore limbs, including the shoulder girdles, were exchanged by dorso-dorsal transplantation, *i. e.,* palms downward and fore arms pointing caudad. After reinnervation had taken place, the grafts moved in perfect coordination, but *always in exactly the reverse sense from what the animal obviously intended.*

1 Summary in P. Weiss, *J. Comp. Neur.*, 1935, **61**, 135.

2 Weiss, P., *Ergebn. d. Biol.*, 1928, **3**, 1.

3 Weiss, P., *Pflüger's Arch. f. d. ges. Physiol.*, 1931, **226**, 600.

4 Unpublished results.

When the animal, to judge from the movements of the normal hind limbs, intended to walk forward, its exchanged fore limbs moved so as to make it walk backward, and upon amputation of the normally progressing hind limbs the backward movement went actually into effect.

This demonstrates that the reflex pattern for the fore limbs, in spite of the awkward consequences it entailed for these animals, had remained unchanged and unadjustable; the left spinal fore limb center continued to discharge a reflex sequence as if for a normal left limb, though such was now missing, and regardless of the fact that the central intention was turned into exactly the opposite effect by being picked up by the grafted right limb with its inverted anatomy. The same holds for the right spinal center in its dealing with a left limb substituted for the right limb.

Although the observations were extended over a period of several months and beyond metamorphosis, the reversed walking and swimming remained unchanged. This fact proves an utter lack of modifiability in the innate patterns of spinal reflex organization in amphibia. An anatomical disarrangement or inversion of the periphery results in functional activities which, in regard to the body, are correspondingly disarranged or reversed. The function remains under all circumstances "organ-correct", whereas it is "body-correct" only as long as the organ retains its normal anatomical relation to the body.

8325 C

## Studies in Water Balance. 1: The Excessive Oxygen Usage Response of Dehydrated Animals to Water and Electrolytes.

HARRY A. DAVIS. (Introduced by E. B. McKinley.)

*From the Department of Surgery, University of Chicago, and Department of Pathology, The George Washington University.*

In certain forms of oedema, particularly those accompanying lipoid nephrosis and certain nutritional states, there is present a marked lowering of the basal metabolic rate. For this reason, it seemed to be desirable to investigate the possible relationship of the total metabolism to water output and to the production of oedema. It has been suggested recently[1] that such a relationship does exist.

[1] Davis, H. A., *Science*, 1935, **81**, 493.

A number of investigators[2-6] have shown that the ingestion of water increases the metabolism of the organism. In this study, we have endeavored to determine from a quantitative viewpoint, the significance of the oxygen consumption rate in the regulation of the output of water.

A series of 50 normal healthy dogs weighing between 7 and 15 kg. was used in this investigation. The animals were anesthetized with sodium barbital in a dosage of 250 mg. per kilo of body weight, in order to prevent movements which might alter the rate of oxygen consumption. Several control metabolic readings upon trained dogs before and after anesthetization showed that sodium barbital had relatively little effect upon the metabolic rate in the dosage recommended. Moreover, the readings were much more consistent in the anesthetized animal than in the unanesthetized. Fluid was administered by vein and by mouth in the form of 0.9% sodium chloride solution, 5% glucose solution and 10% sucrose solution in amounts varying from 2,000 to 4,000 cc. The rate of oxygen consumption was obtained by means of a Krogh respiratory apparatus, readings of 10-minute duration, taken before, during and after the administration of the standard solutions. The urinary bladder was emptied by catheter before each experiment, and the catheter was retained so that the total urine output during the experimental period was obtained. A second series of animals was deprived of water for periods lasting from 5 to 14 days. Food was freely allowed throughout the period of dehydration.

TABLE I.
Influence of Isotonic Sodium Chloride Solutions upon Metabolism of Normal Animal.

| Exp. | $O_2$ Consumption, cc. per min. Before Injection | After Injection | % Increase $O_2$ Consumption | Urine Output cc. |
|---|---|---|---|---|
| 48 | 87.5 | 107.8 | 23.2 | 945 |
| 49 | 66.2 | 79.6 | 20.2 | 950 |
| 46 | 30.0 | 36.2 | 20.6 | 150 |
| 41 | 32.5 | 40.0 | 23.0 | 225 |
| 50 | 77.5 | 99.5 | 28.3 | 960 |

In Table I are shown the results of typical experiments in a series of normal animals. Following the administration of 0.9% sodium

2 Feder, H., *Z. f. Biol.*, 1881, **17**, 531.
3 Wreath, S. R., and Hawk, P. B., *J. Am. Chem. Soc.*, 1911, **33**, 1601.
4 Verzar, F., *Biochem. Z.*, 1911, **34**, 41.
5 Lublin, A., *Z. Klin. Med.*, 1929, **109**, 371.
6 Grollman, A., *Am. J. Physiol.*, 1929, **89**, 157.

chloride solution, there is a gradual increase in the oxygen consumption rate to a point 100-600% above the initial rate. After stopping the ingestion of fluids, the rate returned to the initial level within one hour. In this series, the animals varied considerably in weight. Nevertheless, the increase in the oxygen usage per minute above the initial level differed only from 20% to 28%. In Table I, a definite relationship exists between the height of the oxygen consumption before and after fluid administration and the quantity of the output of urine.

In the dehydrated state the oxygen usage response to water and electrolytes is much greater. This is shown in Table II, where the increase in oxygen consumption rate per minute ranged between 32% and 277%. Transforming these figures into increase per 10-minute intervals, it is seen that the increase varies from 300% to 900% and more. Upon termination of the injection of the solution, the increase in oxygen usage returns to the initial level only after a period of several hours when anhydremia is present.

TABLE II.
Influence of Isotonic Sodium Chloride Solutions upon Metabolism of Dehydrated Animals.

| Exp. | Duration of Dehydration days | $O_2$ Consumption, cc. per min. Before Injection | After Injection | % Increase $O_2$ Consumption | Urine Output cc. |
|---|---|---|---|---|---|
| 34 | 10 | 110 | 141.8 | 38.0 | 225 |
| 35 | 10 | 105 | 145.0 | 38.0 | 100 |
| 37 | 5 | 80 | 105.8 | 32.2 | 500 |
| 38 | 6 | 120 | 452.8 | 277.3 | 600 |
| 43 | 14 | 120 | 171.6 | 43.0 | 400 |

From the observation that the oxygen usage rate returns to the original level despite the fact that the animal still retains the greater portion of the fluid injected in the extravascular tissues, it might be suggested that fluid is more effective as a metabolic stimulant when it is present in the blood stream rather than when it lies in the extravascular tissue spaces. Recent work[7] indicates that fluid is held in the blood stream for longer periods in anhydremia than in normal states. Thus the excessive oxygen usage response of the dehydrated organism to water may be related to this prolonged reservoir action of the blood. Possibly, however, other mechanisms are involved which render dehydrated tissues more susceptible to the metabolic stimulant action of water and electrolytes.

[7] Davis, H. A., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 210.

8326 P

## Studies in Water Balance. 2: Anoxemia Factor in Water Intoxication.

HARRY A. DAVIS. (Introduced by E. B. McKinley.)

*From the Department of Surgery, University of Chicago, and the Department of Pathology, The George Washington University.*

The mechanism of death in intoxication by water is obscure. There is invariably a marked dilution of the blood and certain workers[1, 2, 3] believe that the profound disturbance in the isotonicity of the body fluids which occurs is the cause of death. Others[4] attribute the symptoms to a loss of chloride ions with a resulting alkalosis. Rowntree[5] has pointed out that an oedema of the brain is always present and that, usually, this is the only lesion found in animals dying from the administration of excessive quantities of fluid. The oedema of the brain substance is accompanied, paradoxically enough, by a diminished secretion of cerebrospinal fluid and empty cerebral ventricles. A similar type of cerebral oedema occurs in carbon monoxide poisoning in which an anemic anoxemia is present. In view of this fact, an investigation was undertaken to determine the rôle played by anoxemia in the production of symptoms of water intoxication.

Twelve healthy dogs weighing 8 to 19 kg. were used. Isotonic sodium chloride solutions (0.9%) and isotonic (5%) glucose solution were administered by vein in amounts from 2,000 to 4,500 cc. Metabolic readings were taken before, during and after each experiment at intervals of 10 minutes. Hemoglobin determinations were made by the Newcomer[6] method before and during the administration of fluid. Blood was withdrawn from the femoral artery and vein under oil and the oxygen content was estimated by the Van Slyke[7] manometric gas analysis apparatus at standard intervals.

The following changes occurred during the administration of isotonic fluids. The respiration increased in rate from 20 to 40 and even to 60 respiratory excursions per minute. As the fluid intake increased the breathing became deep, irregular and dyspnoeic in

1 Misawa, H., *Jap. J. M. Sc. Tr., VIII*, 1927, **1**, 355.
2 Helwig, F. C., Schutz, C. B. and Curry D. E., *J. A. M. A.*, 1935, **104**, 1569.
3 Kylin, E., *Z. f. d. ges. exper. Med.*, 1928, **63**, 606.
4 Smith, F. S., Deamer, W. C., and Phatak, N. M., *J. Clin. Invest.*, 1933, **12**, 55.
5 Rowntree, L. G., *J. Pharmacol. and Exp. Therap.*, 1926, **29**, 135.
6 Newcomer, H. S., *J. Biol. Chem.*, 1923, **55**, 569.
7 Van Slyke, D. D., and Cullen, G. E., *J. Biol. Chem.*, 1917, **30**, 289.

type. Later the breathing became shallow. The pulse rate increased from 100 to 150 and even to 200 beats per minute. In the early stages the blood pressure increased 20 to 30 mm. of Hg., but in the later stages it fell below the original level.

In the early stages the oxygen consumption rate increased 50% to 600% or more above the initial level. Later the metabolic rate fell below the original level. The hemoglobin of the blood became much dilated until a critical point was reached which varied from 20% to 50%. In those animals which survived, the dilution of the blood did not go beyond the critical point. However, when this point was passed, the animal invariably died.

The oxygen content of the blood from the femoral artery fell from a normal 18 to 20% to 10% and even to 6% in the later phases of the experiment.

*Oxygen Content of Venous Blood.* The initial values of 13.5% to 15% fell to 6% and finally to 3% in fatal cases. In the later stages of the experiment the oxygen content of the venous blood rose especially when a considerable amount of fluid had collected in the interstitial tissue spaces.

These experiments suggest that the administration of fluids produces an early anemic anoxemia due to a dilution of the hemoglobin of the blood. Later, as fluid accumulates in the interstitial spaces around the arterioles and capillaries, the transfer of oxygen from the blood to the tissues is impeded and a histotoxic form of anoxia is superadded. Landis[8] has shown that anoxemia increases the permeability of the capillaries to fluid, and it is possible that the cerebral oedemas of water intoxication and of carbon monoxide poisoning are explainable upon this basis. It was noted in these experiments that anoxemic symptoms occurred more readily with isotonic glucose than with isotonic sodium chloride solutions. This is in agreement with the work of Loeb[9] upon the relationship between electrolyte concentration and cell respiration.

*Summary.* The correlation of the changes in blood oxygen content, oxygen consumption rate and hemoglobin dilution during the administration of excessive amounts of fluid seems to indicate that the symptoms of water intoxication are those, in part, of an anoxemia.

[8] Landis, E. M., *Am. J. Physiol.*, 1917, **30**, 289.

[9] Loeb, J., *Pflüger's Arch.*, 1903, **97**, 394.

8327 P

## Treatment of Recurrent Tropical Lymphangitis.

JUAN A. PONS. (Introduced by R. F. Loeb.)

*From the School of Tropical Medicine and University Hospital of the University of Puerto Rico, under the auspices of Columbia University.*

Recurrent tropical lymphangitis is very prevalent in Puerto Rico, and the internist is confronted with the problem of treatment for such cases. For a period of one year we have treated 3 series of cases with streptococcus vaccines and streptococcus filtrates. On the results obtained is based this report.

The vaccine used in the first group was prepared from 2 strains (S.T.M. strains $L_9$ and $L_{12}$) of beta-hemolytic streptococci isolated from small ulcers on the feet of each of 2 cases of recurrent lymphangitis during acute attacks, after passage through a mouse and incubation for 48 hours at 37°C. in tryptic digest broth. It contains approximately 1,200,000 dead organisms per cubic millimeter. Fourteen unselected cases have been treated with this vaccine. The injections were given subcutaneously every Tuesday and Saturday over the anterior aspect of the lower third of the thigh of the affected lower extremity. The initial dose was usually 600,000 organisms and the succeeding doses were increased gradually as tolerance developed; the interval between injections was lengthened according to results. The reaction from each injection was carefully recorded at the next visit; its description included a note on the severity and duration of local inflammation, the presence or absence of groin pain and development of systemic symptoms such as chilliness, feverish sensation, headache, general aches and pains. Of 14 cases treated, 7 are eliminated because they give no information as to the result of the treatment. Four abandoned treatment within 3 months. Three have only recently been added to the series. In 3 out of 7 cases, apparent benefit has been obtained from the use of the vaccine. In 3 other cases the vaccine failed completely. In a seventh case there seems to be some improvement which, however, is not very definite.

*Group two* was treated with a vaccine prepared with exactly the same technique as vaccine No. 1, but contains, besides the 2 strains of streptococci isolated from lymphangitis, one strain isolated from erysipelas and another isolated from scarlet fever. Its bacterial content is the same as in vaccine 1 and the same technique of administration was followed. Of 9 cases treated, 4 abandoned treat-

ment within 2 months. In 2 of the other 5, there was an apparent cessation of pyrexial attacks; in a third results so far have been satisfactory; in the 2 remaining cases results have been *nil.*

*A third group* was treated with streptococcus filtrate. A strain of beta-hemolytic streptococcus (STM strain $L_7$) isolated from a case of lymphangitis was grown on streptococcus toxin broth (St. Methods, N. Y. State Dept. of Health, 1927) at 37°C. for 48 hours and placed in the ice box for another 48 hours. Then the broth was passed through a W. Berkefeld filter and bottled; no preservative was added. The bottles were kept in the ice box. Susceptible individuals react locally to small doses of this filtrate when injected intradermally. Injections were given over the anterior aspect of the lower third of the affected thigh, in some cases intradermally, in others subcutaneously. The initial dose was as a rule 0.05 cc. of a 1:100 dilution of the filtrate. This dose was increased gradually until the patient tolerated increasing amounts of the undiluted filtrate. There are 22 cases in this group. Three have been treated for a short time. In 5 cases the treatment has failed completely, in 3 cases the results seem to be beneficial, in 11 cases the results have been highly satisfactory. No special observations have been made on the effects of edema and fibrosis but some patients claim a reduction in the size of the leg. The intradermal administration of the filtrate seems to be more effective than the subcutaneous.

The evaluation of the efficacy of any treatment for recurrent tropical lymphangitis is of necessity difficult. There are no well established clinical criteria by which to measure the benefit or failure of treatment. The frequency and severity of the attacks is the only logical criterium by which results can be judged, yet in this condition spontaneous remissions occur during which there may be no attacks for months or years. The story is often repeated by patients of remissions brought about by a course of neoarsphenamine, by injection of anti-plague or typhoid vaccine, or by taking a decoction of herbs and roots. Only after careful observation for a period of many years can a final word be said concerning apparent favorable results of treatment. In our short observation, however, the filtrate seems to be more effective than the vaccine, specially when given intradermally. The apparently definite rôle of mycotic and other dermatologic conditions of the feet in these cases will be dealt with later.

8328 C

# Relation Between Skin Reactions to Specific Carbohydrate Type I Pneumococcus and Human Blood Groups.

EDWARD S. ROGERS AND HAROLD C. WAGNER. (Introduced by Louis Dienes.)

*From the Medical Research Laboratory and the Anaphylaxis Clinic, Massachusetts General Hospital.*

Tillett and Francis[1] demonstrated the occurrence of a wheal and erythema reaction to the intradermal injection of minute amounts of the homologous type-specific pneumococcus polysaccharide in patients recovering from pneumococcus pneumonia. Subsequent studies by a number of investigators[2] have, in general, confirmed their findings. These authors showed, furthermore, that positive skin tests of this sort were not restricted to cases immediately or even remotely convalescent from pneumonia, but that an appreciable number of supposedly perfectly healthy individuals also gave positive skin tests. Using the Type I carbohydrate the percentage of positive reactions in normal persons was variously reported from 11.6% to 71%. In our own experience prior to the present series positive tests had been encountered in about 70% of normal cases.[3]

Recently Witebsky, Neter, and Sobotka[4] have shown the existence of a close immunological relationship between the Type I Pneumococcus acetyl polysaccharide of Avery and Goebel[5] and the human blood group specific substance A. These authors pointed out, among other observations, that the iso-agglutination of human blood group A corpuscles could be inhibited by Type I Pneumococcus acetyl polysaccharide. They presented strong evidence by means of this method, and also by employing complement fixation and hemolysis inhibition tests, that the relationship was specifically associated with the acetyl polysaccharide and that it was lost in the deacetylated polysaccharide.

These observations upon the relationship between the blood group specific substance A and the Type I Pneumococcus acetyl polysaccharide led to the present attempt to establish a correlation between

1 Tillett, W. S., and Francis, T., Jr., *J. Exp. Med.*, 1929, **50**, 687.

2 Alston, J. M., Galbraith, G. R., and Stewart, D., *J. Path. and Bact.*, 1930, **33**, 845; Finland, M., and Sutliff, W. D., *J. Exp. Med.*, 1931, **54**, 637; Alston, J. M., and Lowdon, A. S. R., *Brit. J. Exp. Path.*, 1933, **14**, 1.

3 Unpublished data, Wagner, H. C.

4 Witebsky, E., Neter, E., and Sobotka, H., *J. Exp. Med.*, 1935, **61**, 703.

5 Avery, O. T., and Goebel, W. F., *J. Exp. Med.*, 1933, **58**, 731.

human blood groups and positive skin tests to the Type I acetyl polysaccharide in so-called normal, non-convalescent persons.

The usual method for intradermal tests was employed. In all cases these tests were done using Type I Pneumococcus acetyl polysaccharide,* the deacetylated form of the same preparation and a normal salt solution control. All readings were made in from 15 to 20 minutes from the time of injection, the presence of a wheal and erythema or erythema alone being interpreted as a positive test. The instances, however, in which erythema was encountered without wheal formation were very few. In a few instances the salt solution controls were positive; such cases have been excluded from the series.

Seventy-eight persons were tested, all but 2 were over 16 years of age. Fifty-six cases (71.8% of the entire group) gave positive tests to both forms of carbohydrate. Only 2 reacted to the acetyl polysaccharide but not to its deacetylated derivative. Of the total number reacting to the Type I carbohydrate 28 persons (48.3%) belonged to either blood groups O or B and, therefore, had blood group specific a-agglutinins in their serums; 46 persons (79.3%) belonged to either blood groups O or A and possessed blood group specific b-agglutinins. Twenty persons failed to give tests to either carbohydrate. Of these, 6 were in blood group O, 7 in group A, 4 in group B, and 3 in group AB.

It seems clear that in this series of studies there is no demonstrable relationship between positive intradermal tests to either the acetyl or deacetylated Type I Pneumococcus polysaccharide and the blood group of the individuals tested. Not only is this shown by the appearance of positive tests in cases of each blood group but also by their failure to appear in individuals in each group as well. That the 20 cases which gave negative tests did not do so because of some refractory state is evidenced by the fact that 14 of them were shown to be sensitive to other antigens commonly used for skin tests. The remaining 6 were not so tested. Furthermore, were there any such relationship, as the work of Witebsky suggests there might well be, a wider separation between the positive reactions to the 2 forms of carbohydrate might be expected, sensitivity resulting from pneumococcus infection being shown by positive reactions to both of the antigens and sensitivity due to the blood group specific a-antibodies being shown by positive reactions to the acetyl polysaccharide alone. Such separation was not encountered.

* The Type I Pneumococcus acetyl polysaccharide employed in these experiments was obtained through the courtesy of Dr. Thomas Francis, Jr., of the Hospital of the Rockefeller Institute for Medical Research.

TABLE I.
Analysis of Results on Basis of Blood Groups of 78 Individuals Studied for Skin Sensitivity to Type I Pneumococcus Polysaccharide.

| Blood Group<br>Serum agglutinins present | O<br>a and b | A<br>b | B<br>a | AB<br>o | No. of cases | % of total cases |
|---|---|---|---|---|---|---|
| Positive skin tests to both acetyl and deacetylated polysaccharide | 19 | 25 | 8 | 4 | 56 | 71.8 |
| Positive skin tests to acetyl polysaccharide only | 1 | 1 | 0 | 0 | 2 | 2.6 |
| Negative skin tests to both acetyl and deacetylated polysaccharide | 6 | 7 | 4 | 3 | 20 | 25.6 |
| Distribution of cases among blood groups | 26 | 33 | 12 | 7 | | |
| Cases having history of pneumonia at some time in the past | 4 | 1 | 1 | 0 | 6 | 7.7 |

It is of considerable interest in regard to the mechanism of the positive reactions in question to note that out of repeated attempts to induce passive transfer of skin sensitivity to the Type I Pneumococcus carbohydrate with the serum from 40 of the positively reacting cases, by the Prausnitz-Küstner technique,[6] only 2 sera were active. In both of these instances the individuals from whom the serum was obtained gave a history of having had pneumonia.

In conclusion it may be stated that in a series of 78 normal individuals tested no correlation could be established between the skin reactivity of these individuals to Type I Pneumococcus specific polysaccharide and their blood groups. It was further shown that sensitivity to the Type I polysaccharide occurring in individuals without previous known pneumococcus infection, could not be transferred passively to the skin of non-sensitive individuals.

8329 C

## Presence of Antibody in Bile.*

JULIAN A. STERLING. (Introduced by I. S. Ravdin.)

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In the course of an investigation of various factors involved in gallstone formation, studies have been carried out on the presence of antibody in hepatic and gall-bladder bile, when demonstrable amounts of antibody were present in the serum.

6 Prausnitz, C., and Küstner, H., *Centralbl. f. Bakteriol.* (Orig.), 1921, **86**, 160.

*Aided by a grant from the Josiah Macy, Jr., Foundation.

Mongrel dogs from our animal house stock were used in these experiments. The procedure consisted, first in determining the agglutinin titer in the serum to staphylococcus *(S. aureus hemolyticus)*, streptococcus *(S. fecalis)*, *B. coli* and *B. typhosum* (flagellate and aflagellate strains). The dogs were then immunized with a polyvalent vaccine, containing the above organisms to a concentration of about three billion per cc. Injection of the vaccine in the course of the immunization was as follows: (Table I.)

TABLE I.

| Day | cc. Injected | Route |
|---|---|---|
| 1 | 1 | Subcutaneously |
| 2 | 2 | " |
| 3 | 2 | " |
| 4 | 2 | Intraperitoneally |
| 10 | 3 | " |
| 11 | 5 | " |
| 12 | 7 | " |
| 13 | 9 | " |

Twenty-five days after the beginning of immunization, the dogs were operated on and a sample of the gall-bladder bile removed, after which cholecystectomy was performed. The common duct was doubly intubated after the method of Elman and McMaster[1] so that specimens of sterile hepatic bile could be recovered. At the same time the antibody titer of the serum was determined.

Numerous studies conducted in this laboratory on the gall-bladder bile and hepatic bile of normal dogs, which are uninfected and show no previous evidence of biliary tract damage, have shown that no significant agglutinin can be demonstrated (a table of normal titers has been omitted, therefore, to conserve space).

Dogs which have been immunized with the polyvalent vaccine prior to common duct intubation have agglutinin present in the serum, in the gall-bladder bile, and in the hepatic bile (Table II).

The agglutinins were present in the highest concentration in the serum. In the hepatic bile, they were of low titer, but the greater concentration found in the gall-bladder bile indicates that antibody is concentrated in that viscus. The ratio of antibody in the hepatic bile to that found in the gall bladder varied from 1:2 to 1:16. The latter figure is interesting in that studies in this laboratory[2] have shown that certain biliary constituents may be found to be 16 times as concentrated in the gall-bladder bile as in the hepatic bile.

[1] Elman, R., and McMaster, P. D., *J. Exp. Med.*, 1925, **41**, 503.

[2] Ravdin, I. S., Riegel, C., and Johnston, C. G., *J. Exp. Med.*, 1932, **56**, 5.

TABLE II.
Agglutinin Titer in Serum, Hepatic Bile, and Gall-Bladder Bile to Bacterial Antigens in Dogs.

| Dog No. | | *Staphylo-coccus* | *Strepto-coccus* | *B. coli* | *B. typhosum* flagellate | *B. typhosum* aflagellate |
|---|---|---|---|---|---|---|
| 19 | Serum | 1:800 | 1:1600 | 1:800 | 1:400 | 1:1600 |
| | Gall-bladder bile | 1:120 | 1:120 | 1:240 | 1:240 | 1:120 |
| | Hepatic bile | 1:60 | 1:60 | 1:120 | 1:120 | 1:60 |
| 20 | Serum | 1:400 | 1:800 | 1:3200 | 1:1600 | 1:3200 |
| | Gall-bladder bile | 1:120 | 1:120 | 1:240 | 1:240 | 1:60 |
| | Hepatic bile | 1:60 | 1:30 | 1:60 | 1:120 | 1:30 |
| 42 | Serum | 1:400 | 1:400 | 1:800 | 1:800 | 1:1600 |
| | Gall-bladder bile | 1:160 | 1:80 | 1:160 | 1:80 | 1:160 |
| | Hepatic bile | 1:20 | 1:10 | 1:10 | 1:40 | 1:10 |

*Conclusions.* Immunized dogs which have developed serum agglutinins to certain strains of the staphylococcus, streptococcus, colon and typhoid bacteria, have those agglutinins present in the hepatic and the gall-bladder bile. The data indicate that antibody is concentrated in the gall bladder.

8330 P

## Lysis of Tubercle Bacilli in Vitro.

WILLIAM STEENKEN, JR. (Introduced by L. U. Gardner.)
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The results of studies on the dissociation of the $H_{37}$ strain of human tubercle bacilli have been reported.[1] The variants found were designated "R" indicating resistant to environment and "S" indicating sensitive to environment. This communication records an interesting lytic phenomenon observed during the study of the effect of ageing $H_{37}$ "R" and "S" variants on gentian violet glycerol egg and plain glycerol egg media of different pH. For clarity it seems advisable to discontinue this usage of the symbols "R" and "S" and to employ them in the usual sense as indicative of a rough or smooth colony structure. To indicate virulence or avirulence the letters (v) or (a) are appended thus "Rv" and "Ra". Since a smooth variant of $H_{37}$ which manifests a typical morphology and virulence has not been obtained this terminology has been adopted to cover

[1] Steenken, W., Jr., Oatway, W. H., Jr., and Petroff, S. A., *J. Exp. Med.*, 1934, **60**, 515

the virulent and avirulent strains of variants having an "R" morphology.

From a dissociated $H_{37}$ culture[1] extreme variants "Ra" and "Rv" were obtained. Each of these variants was seeded in flasks of gentian violet egg and plain glycerol egg media of the following pH 5.0, 5.5, 6.0, 6.2, 6.5, 6.8, 7.0, 7.3, 7.5, and incubated at 37.5°C. in order to follow single colonies. Structural and growth differences of the variants were observed at the end of 6 weeks' incubation. At this time there were no visible colonies on flasks below pH 5.5. The flasks were then returned to the incubator and were examined every 2 weeks over a period of 5 months to observe further changes.

During the first 3 months the cultures of the "Rv" variant showed typical wrinkled or stippled, colonies that were slightly raised and had spreading veil-like peripheries. After a period of 3 or 4 months' incubation at 37.5°C. such colonies on media of pH 6.0, 6.2, and 6.5 became slightly moist and their central portions semifluid with loss of recognizable structure. Extension of the liquefaction toward the periphery resulted in destruction of the original characteristics of the colonies and some of them completely disappeared. The pH of the media at this time was 4.2 in contrast to the original value of pH 6.1.

At the same time small secondary resistant colonies developed which were raised and had large convolutions with clear-cut peripheries. When such colonies were seeded on gentian violet glycerol egg media of pH 5.5 they showed a good growth within 3 weeks with colonies closely resembling the original ones but with no evidence of surface liquefaction. But when such colonies were removed from the media and their under surface examined with a binocular "colony microscope" deep excavations filled with mucoid fluid were discovered.

In smears stained by the Ziehl-Neelsen method the bacillary bodies from these colonies were very pleomorphic ranging from small acid-fast and non-acid-fast dustlike granules to large round or ovoid acid-fast bodies. There were also many acid-fast and non-acid-fast bacilli and a few branching forms. As the colonies grew older the number of granules and round acid-fast bodies progressively increased.

When inoculated into guinea pigs these "Ra" variants produced no spreading macroscopic disease or local tissue damage, but they did create skin hypersensitiveness.

The original "Ra" variant over the same period of time remained practically unchanged except for the development of a slightly

moister surface but there was no evidence of lysis. When transferred to new media the "Ra" colonies grew freely and continued to manifest the same type of colony structure and virulence for the guinea pig.

The same phenomena have been observed with several other "Ra" cultures that were obtained from biopsy and autopsy materials and from sputa. These results will be reported in a later and more detailed publication together with complete data regarding virulence, hypersensitivity and bacillary morphology.

The above observations on the tubercle bacillus parallel those of d'Herelle and Bordet on other bacteria. They demonstrated that the sensitive or "S" type gives rise to a resistive or "R" type which is refractory to the lytic principle.

8331 P

## Motility and Fertilizing Capacities of Fowl Sperm in the Excretory Ducts.

S. STERLING MUNRO. (Introduced by Carl R. Moore.)

*From the Poultry Division, Dominion Experimental Farm, Ottawa, Canada.*

Young[1] showed clearly that spermatozoa of mammals require and undergo a "ripening" process after formation, while being transported through the seminiferous tubules and epididymis during which they acquire not only the power of movement but also the ability to fertilize eggs of their own species.

Certain fundamental differences exist between the anatomy and biology of the avian and mammalian testis which make pertinent the question of just how analogous are the processes underlying sperm physiology in the two classes.

Spermatozoa removed from the testes, epididymides and vas deferens of 30 males of the domestic fowl have been examined for motility when suspended in either Ringer's solution or the diluent recommended by Baker.[2] Using the motility classifications of Moore[3] the activity of sperm from the testis, epididymis and vas deferens was found to be x, xx or xxx and xxxx respectively. There was

1 Young, W. C., *Brit. J. Exp. Biol.*, 1931, **8**, 151.

2 Baker, J. R., *Quart. J. Exp. Phys.*, 1931, **21**, 139.

3 Moore, C. R., *J. Exp. Zool.*, 1928, **50**, 455.

practically no overlapping in motility between the 3 levels. Thus, in general, the attainment of the capacity for movement by the formed spermatozoa in the fowl parallels that demonstrated for mammals; capacity for movement is minimal or only indicated in the testis, increases somewhat in the small epididymis and reaches maximal only in the vas deferens.

When tested by artificial insemination using infertile females a differential fertilizing ability was demonstrated; the fertilizing ability being directly correlated with the power of movement. The data are summarized in Table I.

TABLE I.
**Fertilizing Ability of Sperm from Different Levels of Male Tract.**

| | Hens | | | | | Eggs | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Location | No. of Hens | No. fertile | No. giving Chicks | % fertile | % fertile giving Chicks | No. of Eggs | No. fertile | No. hatch | % fertile | % hatch |
| | | | | Data Secured in 1935. | | | | | | |
| Testes | 45 | 2 | 0 | 4.4 | 0 | 256 | 2 | 0 | 0.78 | 0 |
| Epididymis | 18 | 2 | 1 | 11.1 | 50.0 | 112 | 4 | 1 | 3.57 | 25.0 |
| Vas | 45 | 35 | 29 | 77.8 | 82.9 | 254 | 158 | 111 | 62.20 | 70.25 |
| | | | | Data Secured in 1934. | | | | | | |
| Testes | 14 | 0 | * | 0 | * | 57 | 0 | * | 0 | * |
| Epididymis | 11 | 0 | | 0 | | 46 | 0 | | 0 | |
| Vas | 16 | 7 | | 43.8 | | 60 | 15 | | 25.0 | |

*None of the eggs were incubated beyond 7 days in 1934 and hence no data on hatchability.

Spermatozoa were secured from the respective portions of the reproductive tract immediately after the males were killed, diluted with equal parts of Ringer's or Baker's solutions and introduced directly into the oviduct of the females. The distal end of the oviduct was exposed through the anus by pressure applied simultaneously with the open hand to the posterior and lateral abdominal walls. A glass tube with attached rubber bulb and containing ½ cc. of the sperm suspension was inserted a distance of 1½ to 2 inches into the oviduct. The abdominal pressure was then released, the female retracting the protruded oviduct and at the same time drawing the sperm into the reproductive tract with convulsive movements of the anus which probably involve the distal oviduct.

These results indicate that the sperm of the domestic fowl similar to that of the mammals undergo a process of maturation after formation during which the power of movement and of fertility is attained. Unlike the mammal, however, the sperm do not completely "ripen" in the epididymis, the process being continued during their passage through the vas. This might be expected in view of

the comparative anatomy of the reproductive tract; the epididymis of the fowl being an extremely small organ through which the sperm must pass quickly to the long coiled vas in which they spend the greater part of their time subsequent to morphological maturity and before ejaculation.

8332 C

## Relationship of Precipitin Titers to Gonadotropic Inhibitory Action of Monkey Sera.

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Selye and Collip and their coworkers,[1-4] Twambly and Ferguson,[5] and Meyer and Gustus[6] have demonstrated that the sera of animals injected with gonadotropic hormone contain a substance or substances capable of inhibiting the action of the hormone in test animals. The conditions under which the gonadotropic-inhibitory substance appears in the blood of animals suggest that the mechanism of formation is similar to that involved in the production of antibodies. We have undertaken experiments to ascertain whether or not there is any correlation between the precipitin titer of the serum of monkeys repeatedly injected with the gonadotropic hormone prepared from the serum of pregnant mares and the presence of gonadotropic antagonistic substance.

For this purpose a highly purified preparation of pregnant mare's serum hormone, made by the method of Evans, Gustus and Simpson,[7] was available. Solutions containing 5 r.u.* per cc. of this

1 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 487.

2 Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

3 Selye, H., Bachman, C., Thomson, D. L., and Collip, J. B., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1113

4 Collip, J. B., *J. Mount Sinai Hosp.*, 1934, **32**, 28.

5 Twambly, G. H., and Ferguson, R. S., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 69.

6 Meyer, R. K., and Gustus, E. L., *Science*, 1935, **81**, 208.

7 Evans, H. M., Gustus, E. L., and Simpson, M. E., *J. Exp. Med.*, 1933, **58**, 569.

*A rat unit is that amount of hormone which when injected once daily for three consecutive days produces within 96 hours of the first injection a 5-fold increase in the weight of the ovaries of 21-23-day-old rats.

hormone gave none of the usual chemical tests for protein and reacted only faintly in 1-10 dilution with a potent antihorse serum precipitating serum.

*Macacus rhesus* monkeys, weighing between 2-3 kg., were injected either subcutaneously or intravenously with daily doses of this purified hormone as indicated in Table I. Blood was withdrawn on the days indicated and the amount of serum necessary to inhibit the gonadotropic action of 10 mg. of standard horse serum powder was determined. Precipitin titers were determined in the usual manner, keeping the quantity of monkey serum constant.

TABLE I.

Comparison of Precipitin Titers and Inhibitive Substance in the Sera of Female Monkeys Injected with Gonadotropic Hormone.

| Monkey No. | No. Days Injected | Rat Units Purified Hormone | Days of Exp. | Precipitin Titers: Hormone | Precipitin Titers: Horse Serum | Serum Injected cc. | Wt. of Ovaries of Immature Rats: Exp. mg. | Wt. of Ovaries of Immature Rats: Controls mg. |
|---|---|---|---|---|---|---|---|---|
| 1 | 54 | 245 intrav. | 66 | — | — | 0.5 | 15 | 32 |
| | | | 80 | 16 | 0 | 0.5 | 27 | 64 |
| | | | 136 | 4 | 0 | — | — | — |
| | | | 165 | 16 | 0 | 0.7 | 16 | 43 |
| | | | 212 | 0 | 0 | 1.0 | 17 | 44 |
| | | | 269 | 4 | 2 | 2.0 | 14 | 25 |
| | | | 365 | 2 | 0 | 2.0 | 13 | 25 |
| 2 | 54 | 245 subcut. | 66 | — | — | 0.5 | 11 | 52 |
| | | | 136 | 0 | 10 | 0.5 | 11 | 40 |
| | | | 212 | 0 | 0 | 0.75 | 20 | 44 |
| | | | 365 | 0 | 2 | 1.0 | 15 | 59 |
| 3 | 83 | 629 subcut. | 54 | — | — | 0.5 | 12 | 42 |
| | | | 84 | 0 | 2 | — | — | — |
| | | | 98 | 0 | 0 | 0.2 | 10 | 43 |
| | | | 146 | 0 | 0 | 1.0 | 24 | 44 |
| | | | 305 | 0 | 0 | 1.0 | 24 | 59 |
| 4 | 83 | 415 intrav. | 54 | — | — | 0.5 | 15 | 56 |
| | | | 82 | 10 | 100 | 0.9 | 13 | 56 |
| | | | 98 | 64 | 128 | 0.2 | 13 | 43 |
| | | | 146 | 16 | 0 | 1.5 | 19 | 50 |
| | | | 208 | 2 | 0 | 0.75 | 28 | 50 |
| | | | 316 | 0 | 0 | 2.0 | 25 | 34 |
| 5 | 56 | 390 subcut. | 43 | 0 | 0 | 0.5 | 11 | 40 |
| 6 | 34* | 120 subcut. | 43 | 8 | 2 | 0.5 | 12 | 40 |
| | | | 119 | 0 | 0 | 1.0 | 26 | 48 |
| | 19† | 95 subcut. | 168 | 0 | 0 | 1.0 | 26 | 39 |
| | | | 181 | 0 | 0 | 1.0 | 10 | 65 |
| | | | 273 | 0 | 2 | 2.0 | 13 | 31 |

*5 r.u. day for 11 days; 10 days rest; 5 r.u. day for 13 days.
†After 134 days of rest.

Purified hormone solution and normal horse serum were used as antigens. Normal monkey serum, horse serum and hormone controls were negative throughout the experiments and are not included in the table.

In Table I are shown the data obtained by the precipitin test compared with the inhibitive action of monkey serum on a standard amount of hormone in immature female rats. The serum of monkey 1 showed reactions in low dilutions between the 80th and 212th days of the experiment in the absence of a detectable reaction with horse serum. This reaction decreased as the amount of antagonistic substance in the monkey serum decreased. Such results, however, were not obtained in the case of monkeys 2, 3, 5, and 6. The sera of these animals, with the exception of monkey 2 on the 136th day (10 with horse serum†) and monkey 6 on the 43rd day (8 with hormone) gave no significant reactions with either hormone or normal horse serum. Precipitin titers of monkey 4 serum with both hormone and normal horse serum reached a maximum on the 98th day and decreased thereafter. These titers reached their maximum at the time when the amount of antagonistic substance in the serum was greatest but decreased somewhat more rapidly than the latter.

Monkeys 1 and 4, whose sera gave precipitin titers, were injected intravenously; monkeys 2, 3, 5, and 6, subcutaneously. This may afford a partial explanation for the lack of reactions in the latter group, since intravenous injection usually gives greater antigenic stimulation. Since inhibiting substance was present in the sera of all monkeys in approximately the same amount regardless of the route of injection, it seems likely that the precipitations obtained were non-specific or due to the slight trace of protein present in the hormone preparations.

In order to check this point, 3 additional monkeys were injected subcutaneously with 95, 140, and 265 r.u., respectively of a purified preparation of hormone which had been freed from protein by a method which will be the subject of a future communication by one of us (E. L. G.). This preparation gave no reaction for horse serum protein serologically. Sera of the monkeys so injected gave no significant precipitin reactions with purified hormone, hormone serologically free from horse serum proteins, or normal horse serum, although inhibitory substance for the gonadotropic hormone was present.

† Titer is expressed as highest numerical dilution of antigen giving positive reaction; 0 indicates no reaction with undiluted antigen; —, not determined.

The gonadotropic-inhibitory substance found in the serum of monkeys repeatedly injected with gonadotropic hormone of pregnant mare serum is specific in the antagonistic action and is in this respect similar to an antigen-antibody reaction. Thus we have determined that 2 cc. of serum of monkey 6 (obtained on the 273rd day of the experiment) does not significantly prevent the action of gonadotropic hormone contained in the extracts of human pregnancy urine, whole sheep pituitary gland and whole human pituitary gland. However, the action of the gonadotropic hormone prepared from the serum of pregnant mares was completely inhibited (Table II).

TABLE II.
Serum of Monkey No. 6 Injected in Combination with Various Gonadotropic Hormones.*

| ——Gonadotropic Material—— Kind | Amt. | Amt. of Serum cc. | —Wt. of Ovaries, mg.— Exp. | Control |
|---|---|---|---|---|
| Prolan | 2 r.u. | 2.00 | 23 (5) | 35 (5) |
| Whole sheep pit. | 25 mg. | 2.00 | 34 (5) | 34 (5) |
| " human " | 15 mg. | 2.00 | 108 (4) | 124 (2) |
| Pregnant mare serum | 10 mg. | 2.00 | 13 (5) | 31 (7) |

*Rats injected with .40 cc. serum each of 5 consecutive days; gonadotropic substances on the 3rd, 4th, and 5th days. Autopsy 48 hours after the last injection. Figure in parenthesis indicates number of rats used in determination.

There is evidence that non-specific stimulation of the antibody-forming mechanism results in an increase in antibodies which previously had been produced—the so-called "anamnestic" reaction.[8] Thus monkey 4, whose serum previously contained inhibitory substances, was given 3 weekly injections of typhoid vaccine, to determine whether or not the gonadotropic-inhibitory content of the serum could be increased. The amount of inhibitory substance present in the serum before the injection of typhoid vaccine was very small as determined by the injection of 2 cc. of serum, together with gonadotropic hormone of pregnant mare serum. Although a high typhoid agglutinin titer (5120) was present following these injections, no increase in inhibitory substance occurred. Therefore, non-specific stimulation of the antibody-forming mechanism in this monkey caused no increase in the inhibitory action of the serum.

The results reported in this paper indicate that although the gonadotropic-inhibiting substance developed in monkeys by repeated injections of gonadotropic hormone prepared from the serum of pregnant mares is specific in its action, no reliable indication of its

[8] Topley, W. W. C., "Outline of Immunity," Wm. Wood and Company, Baltimore, 1933, 183-185.

formation or presence is provided by the precipitin test. These results are in essential agreement with those reported by Bachman,[9] who determined the complement-fixation titer of the sera of rabbits repeatedly treated with A P L, although no data showing the concentration of inhibitory substances in the sera of the rabbits were presented.

Ehrlich[10] presented data which showed that by the injections of a hormone preparation ("Präpitan"), rabbits produced specific antibodies in the animal sera which reacted positively with gonadotropic hormone prepared from urine of pregnant women; but not with thyreotropic anterior pituitary gland substance. He did not make any attempt to determine whether the sera contained specific or non-specific gonadotropic-inhibitory substances, nor whether the reactions obtained were due to specific or non-specific antibodies.

It is, of course, difficult in all cases to demonstrate by the presence or absence of *in vitro* reactions the existence of an immune state. For example, individuals following typhoid fever or vaccination occasionally show no demonstrable antibodies against *B. typhosus,* but exposure proves them to be immune. The absence of *in vitro* immunological reaction between the hormone and the monkey serum containing the gonadotropic-inhibitory substance does not necessarily eliminate the possibility that the latter is formed and reacts in a manner similar to that of antibodies.

*Conclusions.* The repeated injection of highly purified gonadotropic hormone prepared from the serum of pregnant mares into female monkeys produced definite quantities of a specific gonadotropic-inhibitory substance and in some cases small amounts of precipitins. There was no predictable correlation between the presence or absence of the precipitins and the gonadotropic-inhibitory substance.

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[9] Bachman, C., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 851.

[10] Ehrlich, H., *Wien. Klin. Wschr.*, 1934, II, 1323.

8333 P

# Virulence, Toxigenic and Fibrinolytic Properties of Streptococci Isolated from Cases of Recurrent Tropical Lymphangitis.

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While studying the biological characteristics of streptococci isolated in the tropics, we have been able to observe a number of strains isolated from cases of recurrent tropical lymphangitis. Fifteen strains from this condition were studied. In determining the fibrinolytic activity of these streptococci the method employed was that recommended by Tillet, Edwards and Garner.[1] The reducible streptolysin was determined by Todd's method as modified by Hodge and Swift,[2] and the toxicity of the strains was studied by intradermic injection in white skinned goats. We also determined hemolysis in the test tube and virulence for mice by the usual laboratory procedures.

The determinations were made immediately after isolation of the organisms and repeated after the organisms had been grown for at least 2 years on 3% rabbit blood agar pH 7.2 and kept in the ice box at 4°C. Two known strains, one isolated from erysipelas and another for scarlet fever, were carried as controls.

None of the strains isolated from lymphangitis produced a reducible streptolysin. All except 2 strains ($L_{13}$ and $L_{21}$) produced fibrinolysin. All except 2 ($L_{13}$ and $L_{21}$) were toxigenic. These 2 strains ($L_{13}$ and $L_{21}$) never produced complete hemolysis in the test tube, did not produce fibrinolysin, and were avirulent for mice. On isolation, 13 out of the 15 cultures were fatal for mice in doses of 0.1 of cc. 18-hour broth culture. Two years after isolation and growth in artificial media, 11 cultures had lost their virulence for mice, yet still produced fibrinolysin, and were toxigenic.

*Summary.* Fifteen strains of streptococci isolated from cases of recurrent tropical lymphangitis have been studied. Thirteen strains were hemolytic, producing a powerful fibrinolysin and were toxigenic and virulent for mice.

1 Tillet, W. S., Edwards, L. B., and Garner, R. L., *J. Clin. Invest.*, 1934, **12**, 47.
2 Hodge, B. E., and Swift, H. F., *J. Exp. Med.*, 1933, **58**, 277.

8334 C

## Comparative Effects of Dinitrophenol and Thyroid on Pituitary-Gonadal Complex of Female Rats.

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Van Horn[1] advanced the view that thyroid feeding in female rats, by raising the metabolic rate, increased the elimination of estrin thereby keeping the estrin level below normal and the animal in a continual state of diestrus. In this way Van Horn believed the pituitary-gonadal complex was affected. The present investigation was undertaken to test this hypothesis using some substance, other than thyroid, which would raise the metabolic rate. Accordingly, dinitrophenol was used. The appearance of the vaginal smear was used as the measure of the estrin level. In addition, the ovaries and the pituitaries were examined microscopically.

Thirty-six normal, adult females were used in this study. A preliminary period of observation for 15 days, during which daily vaginal smears were made, served as a control. Only those animals exhibiting regular 4 to 5 day cycles were chosen. Twelve rats were injected subcutaneously, twice a day, with 25 mg. of dinitrophenol per kilo of body weight. An aqueous solution of 0.5% Eastman's 2-4 dinitrophenol was used, a half gram of sodium bicarbonate being added to each gram of dinitrophenol to facilitate the solution of the latter. With this large dose the rats showed no very marked ill effects; only one rat died. Sixteen rats were divided into 2 equal groups, A and B, and fed desiccated thyroid for varying periods of time, 20 to 42 days. Group A received 0.25 gm. daily and Group B 0.5 gm. The ovaries and pituitaries of the remaining 8 rats, together with glands of 42 rats from another experiment, were studied as controls.

I. *Effect on the Estrous Cycle.* A. Dinitrophenol. (Fig. 1.) An almost constant response is the lengthening of the cycle with an increased period in diestrus. In rats D12 and D16 blood was present in the vagina and it was difficult to secure sufficient material for examination. They appeared to be in diestrus during this time. However, in not one instance did any of the rats receiving dinitrophenol remain permanently in diestrus as did some of the thyroid-fed rats.

1 Van Horn, W. M., *Endocrinology*, 1933, **17**, 152.

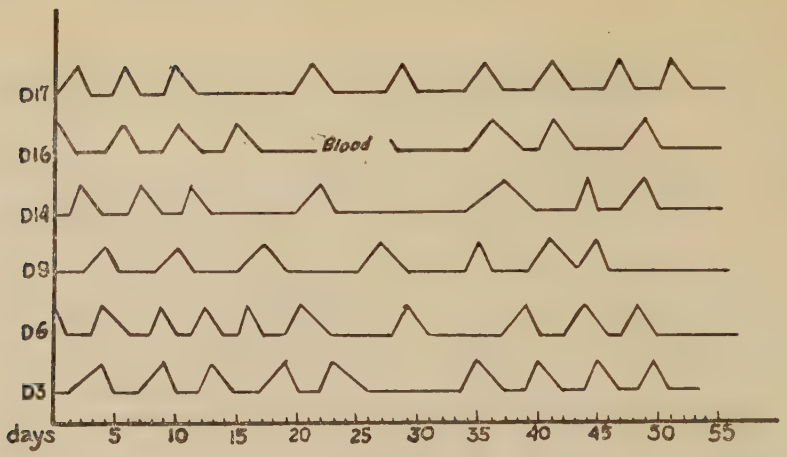


FIG. 1.

Representative estrous curves of 6 normal adult females receiving 0.25 mg. per kg. body weight of 2-4 dinitrophenol, twice daily. Injections began on the fifteenth day. The base corresponds to diestrus, and the peak to estrus (stage 2-3).

B. Thyroid. Following the commencement of thyroid feeding the rats exhibited one or two normal cycles and then went into a continuous diestrus. Rat T34 proved the exception. Although fed 0.5 gm. of thyroid daily for 42 days she maintained normal cycles throughout the course of the experiment.

II. *Effect on the Ovaries.* A. Dinitrophenol. The ovaries were weighed, fixed in Zenker's, and stained with hematoxylin and eosin. The weight and histology were essentially normal, with a tendency for some of the ovaries to have an increased amount of lutein tissue. Rats D12 and D16 received dinitrophenol for 39 days and 42 days, respectively. Blood was present in the vagina of these rats for several weeks. Their ovaries were heavier than normal. In each instance the increase in weight was due to a large hard mass which on cutting exuded a green purulent fluid. Microscopically leucocytes, red blood cells, and cellular debris were noted.

B. Thyroid. In rats receiving 0.25 gm. of thyroid and in those receiving 0.5 gm. for a short period of time the ovaries were heavier and contained numerous corpora lutea, as previously reported by Weichert and Boyd.[2] In rats fed 0.5 gm. thyroid for long periods of time there was a slight loss in weight and a decrease in the number of corpora lutea. Moreover, in this latter group several ovaries showed gross and microscopic signs of cysts. These observations are similar to those reported by Hayashi.[3]

[2] Weichert, C. K., and Boyd, R. W., *Anat. Rec.*, 1933, **58**, 55.

[3] Hayashi, H., *Bull. Acad. de Med.*, 1929, **101**, 115.

III. *Effect on the Pituitary.* A. Dinitrophenol. The glands were cut in serial sections and stained with either Martin's[4] acid fuchsin-methyl blue, or with Severinghaus'[5] modification of the Champy-Kull-Nassanov method. The pituitaries appeared normal in all respects.

B. Thyroid. Our observations are in agreement with Campbell, Wolfe, and Phelps[6] who have already described the anterior pituitaries of hyperthyroid female rats. Since we used a cytological technique to demonstrate the Golgi apparatus and mitochondria we will briefly describe these additional findings. The basophils, which were full of deeply blue stained granules, contained an hypertrophied Golgi apparatus and an increased number of mitochondria. A similar condition of the Golgi apparatus and the mitochondria was observed in many of the enlarged chromophobes. The acidophiles appeared to be diminished in number. Campbell, Wolfe, and Phelps making cell counts found this to be true.

In some respects the pituitaries of thyroid-fed rats resembled the glands of short-time estrin-injected rats (Halpern and D'Amour[7]) in that there was some hyperplasia and hypertrophy of the chromophobes with an increase in the number of mitochondria and the size of the Golgi apparatus. Inasmuch as the ovaries of the hyperthyroid and estrin-injected rats had a predominance of lutein tissue over follicular tissue it seemed quite probable that in both instances the lutein-stimulating hormone of the pituitary had been activated.

*Conclusion.* Although dinitrophenol apparently did raise the metabolic rate it had scarcely any effect on the pituitary-gonadal complex. The pathology in several ovaries and the slightly increased length of the estrous cycles appeared to be due to the toxicity of the drug. This strongly suggests that heightened metabolism *per se* does not lead to an increased rate of estrin elimination, and is, therefore, not sufficient explanation for the marked changes in the estrous cycles, ovaries, and pituitaries of thyroid-fed rats. While this experiment does not completely rule out the effect of increased metabolism on the pituitary-gonadal complex, it does seem to indicate that the thyroid hormone has a specific action on the pituitary and gonads as reflected by the morphological changes observed in these glands.

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4 Martin, T., *Compt. rend. Soc. de Biol.*, 1933, **113**, 1275.

5 Severinghaus, A. E., *Anat. Rec.*, 1932, **53**, 1.

6 Campbell, M., Wolfe, J. M., and Phelps, D., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 205.

7 Halpern, S. R., and D'Amour, F. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 108.

8335 C

# Concerning Enzymic Reactions in Heavy Water. II. Deuterium and the Hydrolysis of Starch.*

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One of us[1] called attention to the contradictory results reported by writers who have investigated various influences of heavy water upon living organisms. It was suggested that studies of numerous biochemical reactions *in vitro,* supplementing investigations upon complex metabolic processes such as respiration, growth, motion, reproduction, etc., in entire organisms, should indicate possible mechanisms involved. Experiments described in the above-mentioned report showed definitely that no retardation of various enzymic reaction rates occurred, but that on the contrary, certain of such processes, notably the hydrolysis of starch by amylase from the crystalline style of the mussel *Mytilus californianus,* were slightly accelerated in high concentrations of $D_2O$. Steacie[2] reports that the inversion of cane sugar by saccharase was retarded by 25% in concentrated heavy water, buffered at pH 4.6, but that the breakdown of the glucoside salicin by emulsin was accelerated to an equal degree in the presence of the pure isotope, buffered at pH (pD?) 4.5.

Hornel[3] cites the work of Moelwyn-Hughes, who found that the catalytic influence of acid in the hydrolysis of cane sugar was greater in $D_2O$ than in ordinary water; also the investigations of Schwartz, who observed that both methyl and ethyl acetates were hydrolyzed more rapidly by 50% in acidic solutions of heavy water than in light water under similar conditions. Hornel's own work on the rate of acid hydrolysis of methyl acetate showed that the catalytic coefficients (assuming that complete dissociation of sulfuric acid took place in both kinds of water) were in the ratio, $K_{D_3O^+}/K_{H_3O^+} = 1.86$ at 15°, and 1.68 at 25°.

* The senior author wishes to acknowledge the generosity of the National Research Council, whose Committee on Grants-in-aid made possible the purchase of the pure heavy water used in these and preceding experiments.

1 Fox, D. L., *J. Cell. and Comp. Physiol.*, 1935, **6**, 405.

2 Steacie, E. W. R., *Z. Physik. Chem.*, 1934, **27**, 6; *ibid.*, 1935, **28**, 236.

3 Hornel, J. C., *Nature*, 1935, **135**, 909.

While, in the earlier work of the senior author, it seemed that the rate of production of maltose from starch was at least slightly enhanced in 99% $D_2O$, it was observed (by the achromic point method) that there was an unmistakable increase in the rate of the first conversion, *i. e.*, that of starch to erythrodextrin, in the same solutions. It was mentioned that further experiments should provide additional information regarding the relative enhancement or retardation of the rate of hydrolysis of "heavy starch", *i. e.*, starch in which the labile, O-linked hydrogens are replaced by deuterium following treatment with heavy water, a process which, according to Brickwedde[4] occurs in such compounds as glucose, sucrose, ethylene glycol, cellulose, etc.

With but a very limited supply of pure heavy water,† on hand, some semi-micro experiments were conducted with a view to illuminating this point. The enzyme was present in the dry pulverized styles of mussels, prepared as described previously (op. cit.).

A series of hydrolyses of hydrated starch by mussel-style amylase, like the chromic (iodo starch) experiments reported in the earlier paper, was repeated, using throughout the series identical quantities of buffer salts (pH 7.0), identical proportions of enzyme, substrate, and water in each system and allowing both enzyme and starch to stand separately, preserved under toluene, in the respective aqueous solutions for an overnight period before mixing and allowing each digestion to proceed at 38°C. The difference in this set of experiments was that the incubations and digestions were carried out in 5 different solutions, *viz.:* 0.0, 24.7, 49.5. 74.2, and 99.2% $D_2O$ instead of only the first and last named, and the rates compared.

Reducing sugars were readily demonstrated with Benedict's reagent in each digest shortly after the beginning.

After incubating for about 2 hours, identical quantities of acidic iodine-potassium iodide reagent were added as a test for residual starch in each system. The most starch (most blue color) appeared in the ordinary water system, the least starch (most brown-purple color, therefore presumably most erythrodextrin) appeared in the 99.2% $D_2O$ system; the other members of the series showed a gradation of intermediate colors in the direction of increasing erythrodextrin and decreasing starch with increasing $D_2O$ concentration of the digestion medium.

The earlier experiments (op. cit.) had revealed that the hydrolysis

[4] Brickwedde, F. G., *J. Wash. Acad. Sci.*, 1935, **25**, 157.

† 99.2% $D_2O$ of certified high purity from the Norsk Hydro-Elektrisk Kvaelstofaktieselskab, Oslo, Norway.

of starch by mussel style amylase in 1% $D_2O$ was not different in rate from the same reaction in ordinary water, all other factors being equal. It was thought possible, however, that if "heavy starch" were first prepared by hydrating the dry material in concentrated heavy water, and allowing sufficient time for labile (*i. e.*, hydroxyl) hydrogen in the starch, to be exchanged for deuterium in the water, this modified polysaccharide might then show a difference in its readiness of breakdown by the enzyme.

Accordingly, 50 mg. of starch were placed into each of a pair of thick-walled Pyrex test tubes containing dry buffer salt from 0.3 cc. of approximately .08 M. phosphate buffer solution of pH 7.0.[5] To one tube was added 0.3 cc. of 99.2% heavy water; into the control tube was placed an equal volume of ordinary twice distilled water. Both tubes were sealed in an oxygen flame and the starch completely hydrated and brought into solution by heating in an oven at 115°C. They were then allowed to stand at room temperature over night. When opened next day, 0.7 cc. of buffer solution was added to each tube to aid in dispersing the gels which had formed on cooling. With both starch and enzyme at 38°C., 5 cc. of a 0.41% style suspension in phosphate buffer solution (pH 7.0) were added to each tube, and the time taken by stopwatch, while the resulting mixtures were allowed to incubate in a glycerine bath maintained at the above temperature; 1 cc. samples were withdrawn from time to time and titrated in the regular manner, using Benedict's quantitative copper reagent, for maltose formed.

Reference to the curves in Fig. 1 shows that the hydrolytic reaction had reached equilibrium far sooner in the case of the "heavy starch" in approximately 5% heavy water than in the control; the 120 minute point was the first in which as great an amount of maltose was measured in the control digest as was found in the $D_2O$ system after the first 10 minutes. The curves would indicate an initial difference in rate of hydrolysis of three or four fold. It will be noted that this experiment, which reveals different results from an earlier one (op. cit.) in which 1% $D_2O$ allowed the hydrolysis of starch to take place simultaneously with the same reaction in the control, was conducted in a very different manner in that, instead of merely incubating the previously hydrated substrate with the dilute $D_2O$ solution as formerly, the substrate was in this case originally hydrated in the *hot concentrated* isotope water. The protium-deuterium exchange between water and certain other substances,

[5] Clark, W. M., The Determination of Hydrogen Ions, 3rd ed., 1928, Williams and Wilkins, Baltimore.

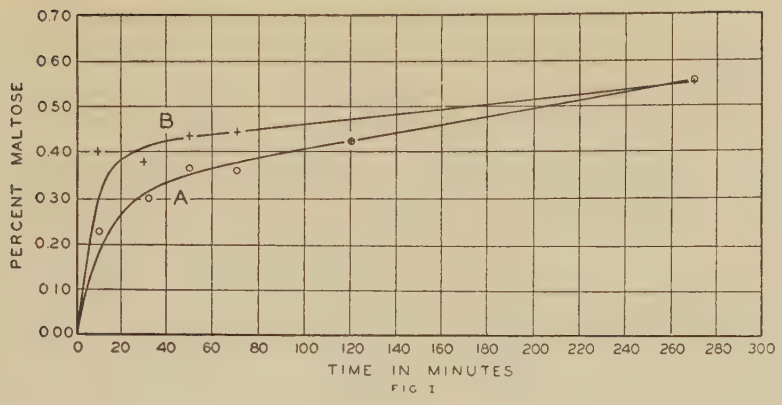


FIG. 1.

Hydrolysis of starch (previously hydrated in respective kinds of water at 115° C.)
Concentration of buffer salts, ca .08 M, pH 7.0; temp. 37-38° C.
Starch concentration 0.83%
Style concentration 0.33%
A (circles); starch hydrated and hydrolized in ordinary water.
B (crosses); starch hydrated in 99.2% $D_2O$.
hydrolized in 5% $D_2O$.

according to Brickwedde,[4] occurs readily. See also Polanyi.[6]

The addition of the same quantity of iodine reagent to the residue in each tube at the end of the experiment yielded at first similar appearing brown-red solutions but later showed the deep blue to blue-purple color of residual starch in the control tube and the red-purple of predominating erythrodextrin in the heavy water tube.

From the few experiments permitted by the limited supply of heavy water on hand, it is provisionally concluded that starch which has been allowed to become hydrated with heavy water, or which may have exchanged some of its labile protium for deuterium, is more readily hydrolyzed by this enzyme, other conditions being the same, than is starch of ordinary history.

A very interesting projection of such experiments as these would be a test of the relative rates of starch synthesis (in light) and starch hydrolysis (in darkness) by algae grown in dilute $D_2O$ on the one hand and in ordinary water on the other.

[6] Polanyi, M., *Nature* (Supplement), 1934, **135**, 19.

8336 C

# Interconversion of Ketose and Aldose Sugars in Dilute Aqueous Solution.

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The present article contains a study of certain changes produced when dilute aqueous solutions of various sugars are boiled. Interest in the subject was aroused because it was observed that in a solution of glucose so treated fructose was apparently produced.[1] Although the interconversion of glucose, fructose, and mannose in alkaline solution has been repeatedly studied since Lobry de Bruyn and Van Eckenstein first observed the phenomenon,[2] results of quantitative studies similar to those reported do not seem to be available.

The technical procedure was as follows: Pure glucose from the Bureau of Standards was dried over calcium chloride and dissolved in distilled water. Seventy-five cc. of a solution so prepared were boiled on an electric hot plate under a reflux condenser. At intervals the flask containing the solution was rapidly cooled in ice water and a sample removed for analysis. The solution was then reheated rapidly and the boiling continued. The changes in the concentration of reducing substances were determined by the copper reduction method of Benedict[3] and variations in the intensity of the resorcinol reaction by the technique described by Roe[4] for the determination of fructose in blood. The purest glucose and fructose obtainable were used as standards. The reaction values were estimated colorimetrically at room temperature. Pure commercial mannose and galactose prepared for the bacteriological laboratory were used in some experiments.

Water solutions of glucose were studied first. It soon became evident that several conditions affected the composition of the boiled mixture. One of these was the concentration of glucose initially present. When the solution was very dilute the maximum concentration of "fructose" was generally reached after boiling for 4 hours, but in stronger ones such a value was not reached in very much

[1] Hubbard, R. S., and Garbutt, H. R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 986.

[2] Armstrong, E. F., The Simple Carbohydrates and the Glucosides, second edition, London, 1912, p. 40.

[3] Benedict, S. R., *J. Biol. Chem.*, 1925, **64**, 207.

[4] Roe, J. H., *J. Biol. Chem.*, 1934, **107**, 15.

longer periods. Results of analyses made after 6 hours are given in Table I. It can be seen that the concentration of "fructose" present at this time varied with the concentration of glucose initially present, but was not parallel to it. Since there was very little difference between the percentage composition of the products obtained by boiling 0.02 and 0.1% glucose in a neutral buffer mixture (Table) it seems possible that variations in acidity may have been one of the causes of this lack of parallelism.

TABLE I.*

Formation of "Fructose" on Boiling Aqueous Solutions of Glucose for Six Hours.

| Initial concentration of pure glucose mg./100 cc. | "Glucose" after boiling mg./100 cc. | "Fructose" formed by boiling[1] mg./100 cc. | Reaction pH | Proportion of "total sugar" as "fructose" % |
|---|---|---|---|---|
| 5 | 4.05 | 1.36 | — | 36 |
| 10 | 8.70 | 2.38 | 6.7 | 27 |
| 20 | 18.6 | 5.30 | 6.8 | 27 |
| 40[2] | 40 | 6.72 | — | 17 |
| 100 | 98 | 10.9 | — | 11 |
| 1000 | 1000 | 49.6 | 6.5 | 5 |
| 2000[3] | 2000 | 62.0 | 5.9 | 3 |
| 20[4] | 18.4 | 5.60 | 7.0 | 30 |
| 100[4] | 101.4 | 29.8 | 7.0 | 29 |
| 20[5] | 19.5 | 4.18 | 6.9 | 21 |

*Under "glucose" and "total sugar" are given the reducing power in terms of glucose. Under "fructose" is given a quantitative measure of the resorcinol reaction as fructose. Reaction values were determined at room temperature.

[1]Corrected for the resorcinol reaction given by the unboiled glucose solution.

[2]Results after boiling for 4 hours.

[3]Nitrogen gas led through the solution continuously during the experiment.

[4]Phosphate solution in M/15 concentration giving a pH of 7.0 present in these experiments. Boiled only 4 hours.

[5]One per cent sodium fluoride present in this experiment.

A decrease in reducing power was found in the dilute but not in the stronger solutions studied. Since the reducing power of the carbohydrates probably present in such a mixture varies (fructose gave results approximately 6% higher, and mannose results 12% lower than glucose by the method used), this diminution in reducing power might have been due to the same type of molecular rearrangement as leads to the production of a ketose. The authors believe, however, that some destruction of one of the sugars in the mixture must have taken place or some significant change in the reducing power of the one and 2% solutions would have been found.

It will be noted that the formation of ketose occurred in these experiments at a reaction decidedly more acid than pH 12.3—the value which corresponds to the acidic dissociation exponent of glu-

cose.[5] Although the reaction at 100° is not correctly given by measurements at room temperature, it seems to the authors that even those values must have been distinctly less alkaline than the one indicated by the constant given above. It seems possible, however, that some acidic dissociation of the sugar was produced by heat.

The results of an experiment in which sodium fluoride was added to the glucose are included in Table I. Because the reaction was somewhat more acid during the course of this experiment than during that of the others recorded, it does not seem proper to emphasize the significance of the relatively slight destruction of sugar noted in the presence of this salt.

Sugars in 0.02% solution seemed suitable for study by the methods selected, and glucose, fructose and mannose were treated in this concentration in various ways. Results with mannose were similar to those shown in Table I, but the changes were less marked and less consistent. Only 10% of the total reducing substance was in the form of a ketose after 6 hours' boiling. Determinations of the reducing power gave very irregular results, probably because destruction and conversion into a substance with greater reducing power than mannose (glucose?) occurred simultaneously.

TABLE II.*

Dilute Aqueous Sugar Solutions Boiled in the Presence of Oxygen and Nitrogen.

| Time of boiling | ——0.02 % glucose studied—— "Glucose" | "Fructose" | Reaction | Time of boiling | ——0.02 % fructose studied—— "Glucose" | "Fructose" | Reaction |
|---|---|---|---|---|---|---|---|
| hr. | mg./100 cc. | mg./100 cc. | pH | hr. | mg./100 cc. | mg./100 cc. | pH |
| | Oxygen gas led continuously through the solutions. | | | | | | |
| 0 | 20 | 0.25 | 5.8 | 0 | 21.3 | 20 | 5.9 |
| 2 | 19.9 | 3.63 | 6.8 | 2 | 19.1 | 17.6 | 5.9 |
| 4 | 19.1 | 5.20 | 6.8 | 4 | 18.5 | 15.7 | 6.1 |
| 6 | 17.9 | 6.15 | 6.9 | 6 | 17.4 | 13.7 | 6.7 |
| | Nitrogen gas led continuously through the solutions. | | | | | | |
| 0 | 20 | 0.2 | 6.0 | 0 | 21.3 | 20 | 6.0 |
| 2 | 20 | 4.0 | 7.2 | 2 | 18.6 | 17.0 | 5.6 |
| 4 | 19.1 | 6.1 | 7.2 | 4 | 18.0 | 15.0 | 5.8 |
| 6 | 18.3 | 6.5 | 7.0 | 6 | 17.5 | 13.3 | 6.4 |

In Table II are given details of representative experiments with glucose and fructose. In these experiments oxygen and nitrogen were bubbled at a slow rate through the boiling solution throughout the 6-hour period. There was probably some loss of water vapor with the escaping gas, but the results were not significantly different from those of other experiments in which gas was not used.

The results of the experiments with the 2 gases are seen to be

[5] Clark, W. M., The Determination of Hydrogen Ions, third edition, Baltimore, 1928, p. 678.

almost exactly alike; such differences as exist between the sets of quantitative analyses are certainly not greater than can be attributed to the differences in reaction found. The results upon each of the sugars will be separately discussed.

Glucose when boiled gave rise to a substance giving the resorcinol reaction. The rate at which this substance was produced was most marked during the first part of the experiment. After 4 hours equilibrium had been approximately reached in the experiment with nitrogen. There was no measurable decrease in reducing power during the first 2 hours of boiling. Thereafter a significant change could be demonstrated. All of the substances in the boiled mixture were fermentable when treated by massive inoculation with yeast cells for short periods. This observation was confirmed on other similar preparations. The non-fermentable sugar "glutose"[6] could not be demonstrated.

Fructose when boiled showed a loss both in the reducing power and in the intensity of the resorcinol reaction. These losses were regularly greater in the latter factor. The differences between the changes can properly be interpreted as resulting from a conversion of fructose into a sugar, or mixture of sugars, which do not give the ketose test. The loss in reducing power, which was shown during the first period of the experiment, the authors interpret as a change of part of the sugar into some non-reducing substance. The boiled mixture after fermentation with yeast did not give either a reducing or resorcinol reaction.

When the results with the 2 sugars are compared it is seen that the proportion between ketose and total sugar was lower when glucose was used than when fructose was studied. This suggests that a ketose was more readily formed from glucose than was an aldose from fructose. Since the reaction values in the experiments upon the 2 sugars were quite different, great emphasis should not be placed upon this contrast.

A conversion of glucose, fructose and mannose into mixtures of fermentable compounds which show reducing and resorcinol reactions can be demonstrated in dilute aqueous solutions. It seems probable that these changes closely resemble those observed in alkaline solutions, although the reaction did not approach the pH value of the acid dissociation constant of glucose (12.3). Some loss in reducing power was also found. The changes were not measurably influenced by oxygen.

[6] Benedict, E. M., Dakin, H. D., and West, R., *J. Biol. Chem.*, 1926, **68**, 1.

8337 C

## Changes in Composition of Dilute Buffered Carbohydrate Solutions Produced by Boiling.

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Because it seemed probable that variations in reaction had affected the results obtained when dilute aqueous solutions of carbohydrates were boiled,[1] the investigation was extended to include buffered solutions. Phosphate mixtures were chosen because we wished to study solutions at approximately neutral reactions. Since it seems possible that phosphate may have some specific effect upon the transformation of aldoses into ketoses[2] experiments with acetate buffer mixtures were also carried out. The effect of oxygen and nitrogen upon the reaction and the fermentability of the compounds in the boiled solutions were also investigated. The technique is described in the preceding paper.

In Table I are given the results of experiments in which the phosphate content of dilute glucose solutions was kept constant and the degree of acidity varied. It is evident that both the speed of formation of "fructose" and of decrease in the reducing power varied with the reaction: they were highest in the most alkaline and lowest in the slightly acid ones. It is worth noting that a measurable formation of "fructose" could be demonstrated when the reaction was 6.0 pH. The change at a pH of 7 resembled that found in simple aqueous solutions, for diminution in the reducing power was very slight during the first 2 hours of boiling and quite marked during the latter part of the experiment when ketose was already present in relatively high concentration. When the pH value was 7.8 a marked ketose formation accompanied the rapid destruction of sugar.

In Table II are presented the results obtained when mixtures of sodium acetate and acetic acid giving pH values over the range shown in Table I were used. The difference between the 2 sets of results was slight and probably not significant. As far as can be told neither salt had a specific effect upon the formation of ketose, but at

[1] Garbutt, H. R., and Hubbard, R. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 270.

[2] Smith, M. L., *Biochem. J.*, 1932, **26**, 1467.

TABLE I.*

Effect of Boiling 0.02 % Glucose in M/15 Aqueous Phosphate Solutions.

| Time of boiling hr. | — Buffered to 5.0 pH[1] — Glucose mg./100 cc. | Fructose mg./100 cc. | — Buffered to 6.0 pH — Glucose mg./100 cc. | Fructose mg./100 cc. | — Buffered to 7.0 pH — Glucose mg./100 cc. | Fructose mg./100 cc. | — Buffered to 7.8 pH — Glucose mg./100 cc. | Fructose mg./100 cc. |
|---|---|---|---|---|---|---|---|---|
| 0 | (20)[2] | 0.25 | 20 | 0.2 | 20 | 0.2 | 20 | 0.2 |
| 2 | — | 0.25 | 20 | 0.95 | 19.8 | 4.63 | 14.7 | 6.76 |
| 4 | — | 0.25 | 19.6 | 1.67 | 18.4 | 5.8 | 9.75 | 3.67 |
| 6 | — | 0.25 | 19.6 | 2.22 | 17.5 | 5.7 | 6.9 | 2.22[3] |

* Under "glucose" is given the reducing power in terms of glucose, and under "fructose" a quantitative measure of the resorcinol reaction in terms of fructose.

[1] Approximate reading; sodium di-hydrogen phosphate alone present; reaction remained unchanged throughout the experiment.

[2] High acidity of the solution prevented a quantitative determination.

[3] The reaction of this specimen, as determined at room temperature, was 7.6 pH. The reaction of no other specimen differed from those indicated in the column headings by as much as 0.1 pH when so determined.

TABLE II.

Effect of Boiling 0.02 % Glucose in 0.2 N Aqueous Acetate Solutions.

| Time of boiling hr. | —— 6 cc. 0.2 N acetic acid —— Glucose mg./100 cc. | Fructose mg./100 cc. | pH | —— 0.2 cc. 0.2 N acetic acid —— Glucose mg./100 cc. | Fructose mg./100 cc. | pH | ———— No acetic acid ———— Glucose mg./100 cc. | Fructose mg./100 cc. | pH |
|---|---|---|---|---|---|---|---|---|---|
| 0 | 20 | 0.2 | 5.8 | 20 | 0.2 | 7.0 | 20 | 0.2 | 7.8 |
| 2 | 20 | 0.97 | 5.8 | 20 | 4.67 | 7.0 | 15.3 | 4.63 | 7.3 |
| 4 | 20 | 1.96 | 5.8 | 17.4 | 5.88 | 7.0 | 11.8 | 4.00 | 7.1 |
| 6 | 19.8 | 2.80 | 5.8 | 15.7 | 5.70 | 7.0 | 9.5 | 3.64 | 7.2 |

All reaction values were determined at room temperature.

The amount of acetic acid in 100 cc. of each buffer mixture used is shown in the appropriate column heading.

a pH of 7 the destruction of glucose was perhaps a little more rapid in the acetate than in the phosphate solution.

In Table III results with sugars other than glucose are given. When an allowance is made for the difference in reducing power shown by the 2 sugars, figures given by mannose were practically identical with those obtained in the study of glucose. Galactose also showed a change of a similar type, but the rate of formation of ketose was apparently less rapid than that found with the other aldoses. Little or no emphasis can be placed upon this difference. The sugar was not fructose, for it was not fermentable, and data upon the relative intensity of the resorcinol reaction when various ketoses are treated by Roe's technique are not available. The sugar produced was probably tagatose.[3]

The results in Table I showed that rather slight variations in acidity had a marked effect upon the production of a ketose from glucose. The results obtained in these buffered solutions should therefore furnish fairly satisfactory material for comparing changes in glucose and fructose with each other. The rates of destruction, shown by loss in reducing power, will be discussed first. It is evident that, as in the experiments on water solutions, destruction did not begin in the solutions of the aldoses (glucose and mannose) until the 3rd or 4th hour of the experiment when ketose was already present in fairly high concentration. Fructose, however, showed destruction from the beginning of the experiment. These results suggest that glucose was first converted into a ketose and then this ketose was destroyed.

Formation of a reducing, fermentable substance from fructose is quite clearly shown by a comparison of the reducing and resorcinol reactions found after boiling. The change into an aldose was much more marked than that found in the water solutions. In the phosphate buffer giving a pH of 7 the composition of the mixtures produced by boiling fructose, glucose, and mannose for 6 hours were quite similar. Thirty-six per cent of the total reducing substances derived from fructose were still in the ketose form at that time, while glucose and mannose gave mixtures containing respectively 33 and 34% of the "total sugar" in the form of "fructose". Since 2 separate factors—the change in the sugar and the destruction of reducing substances—have cooperated in producing these results the authors do not feel justified in placing great emphasis on the finding.

[3] Armstrong, E. F., The Simple Carbohydrates and the Glucosides, third edition, London, 1913, p. 47.

TABLE III.

Dilute Solutions of Various Sugars Boiled in Aqueous M/15 Phosphate to Give pH of 7.

| Time of boiling hr. | 0.02% fructose | | | 0.02% mannose | | | 0.02% galactose | | |
|---|---|---|---|---|---|---|---|---|---|
| | Glucose mg./100 cc. | Fructose mg./100 cc. | pH | Glucose mg./100 cc. | Fructose mg./100 cc. | pH | Glucose mg./100 cc. | Fructose mg./100 cc. | pH |
| 0 | 20.6 | 18.5 | 7.0 | 18.8 | 0.2 | 7.0 | 20 | 0.1 | 7.0 |
| 2 | 17.0 | 10.6 | 7.0 | 18.7 | 4.65 | 7.0 | 19.5 | 3.16 | 7.0 |
| 4 | 15.6 | 6.68 | 7.0 | 18.3 | 5.48 | 7.0 | 19.0 | 3.72 | 7.0 |
| 6 | 13.3 | 4.88 | 7.0 | 16.7 | 5.71 | 7.0 | 16.9 | 3.72 | 7.0 |

Reaction values were determined at room temperature.

TABLE IV.

Dilute Sugar Solutions in Phosphate Boiled in the Presence of Oxygen and Nitrogen. 0.02 % Sugar in M/15 Phosphate to Give a pH Value of 7.0 Studied.

| Time of boiling hr. | Experiments with oxygen | | | | Experiments with nitrogen | | | |
|---|---|---|---|---|---|---|---|---|
| | Glucose used | | Fructose used | | Glucose used | | Fructose used | |
| | Glucose mg./100 cc. | Fructose mg./100 cc. | Glucose mg./100 cc. | Fructose mg./100 cc. | Glucose mg./100 cc. | Fructose mg./100 cc. | Glucose mg./100 cc. | Fructose mg./100 cc. |
| 0 | 20 | 0.15 | 20 | 20 | 20.5 | 0.15 | 21.0 | 20.2 |
| 2 | 17.9 | 3.64 | 12.1 | 7.61 | 20.5 | 3.36 | 19.6 | 12.3 |
| 4 | 12.7 | 2.72 | 6.65 | 3.28 | 20.3 | 4.9 | 17.9 | 10.3 |
| 6 | 7.6 | 1.6 | 4.83 | 1.2 | 19.3 | 5.8 | 16.5 | 8.0 |

The reaction of each specimen was found to be 7.0 pH when determined at room temperature.

The results given in Table IV are those obtained when gases were bubbled through boiling solutions containing phosphate to give a neutral reaction. They should be compared, not only with others in this article, but also with those in Table II of the preceding one. In the nitrogen experiment the rate of formation of ketose from glucose was approximately the same as when the gas was not used. It is evident, however, that the rate of decrease of the reducing power shown by both sugars was less when this gas was used than in the control experiment, and that the amount of ketose still present in the fructose solution after 6 hours boiling was approximately twice as great in the former as in the latter. The authors interpret these figures as supporting their thesis that loss in reducing power represents destruction of sugar, or, more properly, conversion of sugar into some non-reducing substance, and that this change is largely at the expense of the ketose. The complicated nature of the reactions in these solutions of course makes it impossible to decide whether this represents the only, or even the main, cause of the loss of reducing power found.

A comparison of the composition of the mixture obtained by boiling these 2 sugars for 6 hours is interesting. Fructose still contained 48% of the total reducing substances in the form of a ketose at that time, while glucose, after similar treatment, gave a mixture with only 30% in that form. It is evident that the agreement shown by the figures discussed above was markedly influenced by differences in the rates at which the 2 sugars are destroyed. The significance of the figures as an expression of an equilibrated mixture seems therefore questionable.

The effect of running oxygen through the boiling mixture was striking. The reducing power of both the glucose and the fructose solutions decreased rapidly; fructose showed the greater change. Since such a result was not found when a water solution containing no phosphate buffer was treated in a similar way it seemed possible that the phosphate had had a specific effect upon the change. The probability that such a specific effect existed was strengthened by an experiment in which glucose was buffered to a pH of 7, with an appropriate acetate mixture and boiled in the presence of oxygen gas. The results were almost identical with those obtained in a similar solution not treated with gas. (Table II.) The composition of the mixture at the end of 6 hours was: "glucose" 15.7 mg./100 cc.; "fructose" 5.2 mg./100 cc.; reaction 7.0 pH.

The product obtained by boiling these dilute sugar solutions in the neutral phosphate buffer mixture was fermented by treating 5 cc.

of the solution with one cc. of packed, washed yeast cells for 10 minutes. The initial solutions of glucose, fructose and mannose showed no reducing or resorcinol reaction after one treatment of this kind; the boiled solutions, however, regularly showed both reactions after repeated treatments, suggesting that some non-fermentable sugar (glutose?[4]) was present. The average values were: From glucose: 1.5 mg. "glucose" per 100 cc.; 0.59 mg. "fructose" per 100 cc. From fructose: 2.2 mg. "glucose" per 100 cc.; 1.2 mg. "fructose" per 100 cc. From mannose: 1.5 mg. "glucose" per 100 cc.; 0.7 mg. "fructose" per 100 cc. Similar analysis of boiled 0.1% solutions after prolonged incubation with yeast gave similar results; the actual amounts of "glutose" were larger, but the relationship between the amounts obtained from glucose and levulose, and the relative intensities of the reducing and resorcinol reactions were similar.

When dilute solutions of hexoses containing buffers to give reactions which were neutral, or which varied only slightly from a pH of 7 were boiled the following effects were observed: a molecular rearrangement similar to that shown by alkaline solutions, with a formation of fructose from glucose and mannose, and of a mixture of aldoses from fructose took place; a formation of fructose from glucose was demonstrated at a pH of 6; simultaneously with these changes there occurred a destruction of sugar, which was shown by a decrease in the total reducing power; this destruction of sugar was most marked in the slightly alkaline solutions, and seemed to take place largely at the expense of the ketose present; the change of one form of sugar into another was independent of the nature of the salt mixture used in buffering the solution; phosphate increased markedly the rate of destruction of sugars by oxygen; a small amount of a non-fermentable sugar was formed in solutions of pH 7.

[4] Benedict, E. M., Dakin, H. D., and West R., *J. Biol. Chem.*, 1926, **68**, 1.

8338 C

## Vitamin A Requirements of Growing Puppies.

W. O. FROHRING. (Introduced by H. Goldblatt.)

*From Private Laboratory, Cleveland, Ohio.*

While observations on vitamin A deficiency in the dog have been reported by several investigators,[1-4] no attempt to determine the dog's minimum requirement of vitamin A has appeared in the literature. This report deals with such a study.

The synthetic vitamin A-free milk previously described[5] was used in these experiments. In that report[5] illustrations are given of xerophthalmia produced with this synthetic milk and cured by the addition of Carotene to this diet.

In this group of puppies herein reported, the curative method was used. All the puppies used were started on the vitamin A-free ration at weaning, or at most, 1 or 2 weeks later. It has been found highly important to rid the animals of any intestinal parasites at the beginning of the experiment. All the animals were devocalized under deep anesthesia. The total number of puppies used was 31.

The first curative levels used were based on the curative dose per 100 gm. of body weight of the albino rat. The negative results obtained with these levels resulted in the loss of a large number of the puppies. With considerably higher levels, a growth response and improvement in the condition of the animals were obtained.

The results of various levels in the largest litter are given in the chart. The minimum curative dose which effected definite increase of weight was 20 U.S.P. units per 100 gm. of body weight per day. The weight used for these figures is the weight at the beginning of the curative dose. The maximum curative dose used in these experiments was 70 U.S.P. units per 100 gm. of body weight per day.

While the daily minimum curative dose seems high in comparison with the rat, no blue units were found in any of the livers of the test animals killed at the end of the experiment. Even the control getting 7,000 U.S.P. units daily from the beginning shows a very low

1 Steenbock, H., Nelson, E. M., and Hart, E. B., *Am. J. Physiol.*, 1921, **58**, 14.

2 Ralli, Elaine P., Pariente, Arthur, Flaum, Gerald, and Waterhouse, Alice, *Am. J. Physiol.*, 1933, **103**, 2.

3 Stimson, A. M., and Hedley, O. F., *U. S. Public Health Reports*, 1933, **48**, 17, 445.

4 Olcott, H. S., Proc. Soc. Exp. Biol. and Med., 1933, **30**, 767.

5 Frohring, W. O., Proc. Soc. Exp. Biol. and Med., 1935, **32**, 1021.

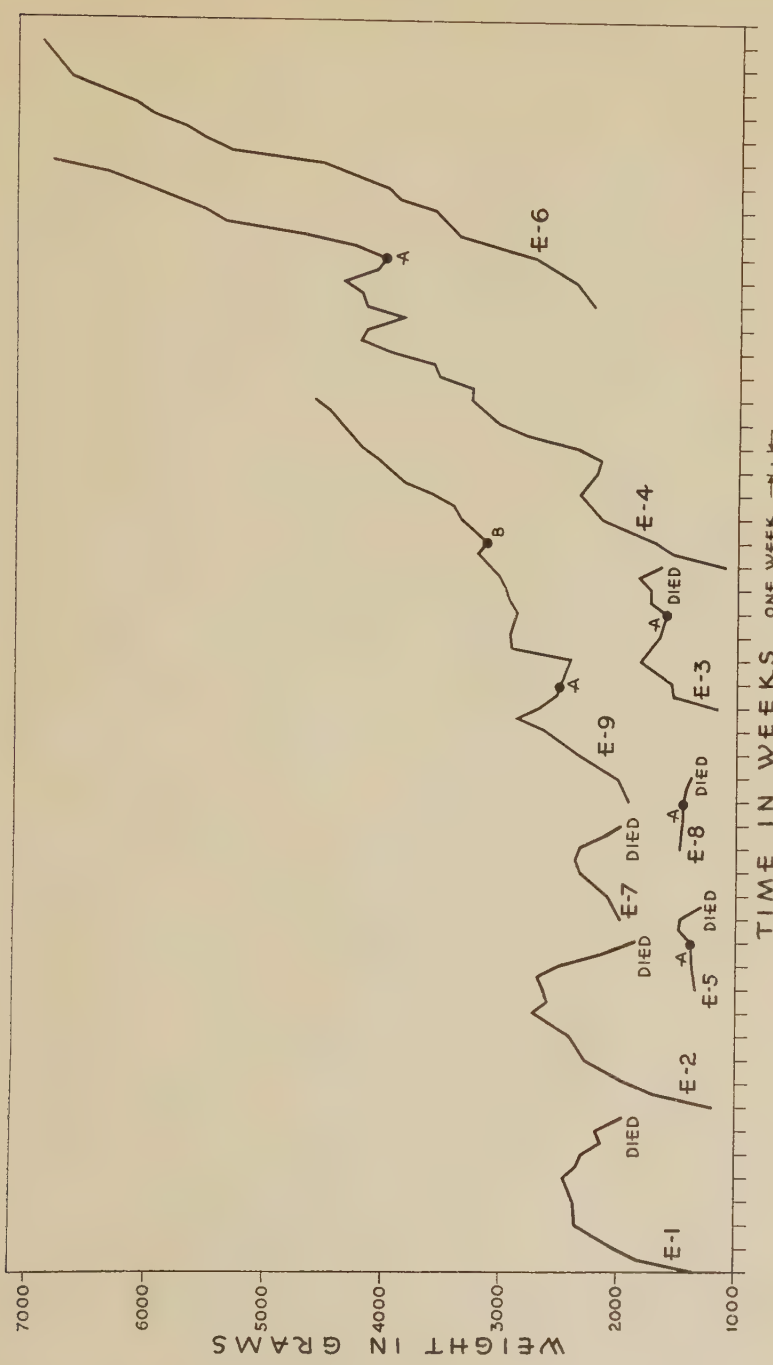



FIG. 1.

E-5 was given 210 U.S.P. units per day, starting at "A." E-8 was given 280 U.S.P. units per day, starting at "A." E-3 was given 420 U.S.P. units per day, starting at "A." E-9 was given 700 U.S.P. units per day, starting at "A," and 1400 U.S.P. units per day, starting at "B." E-4 was given 2800 U.S.P. units per day, starting at "A." E-6 was a control and was given 6500 U.S.P. units per day from the beginning to the end of the experiment. Carotene, as Pro-Vitamin A in cottonseed oil, was used as a source of vitamin A activity in all of the above animals.

reserve of 2 blue units per gram of liver. On the basis of the average daily weight and the total number of units of vitamin A given during the test period, it averages 114 U.S.P. units per 100 gm. of body weight per day.

Loss of appetite was one of the first signs of vitamin A depletion. Eye infections appeared in the animals given sub-minimum doses. A peculiar divergent strabismus was noted in several of the depleted animals which was definitely improved but not completely cured by the minimum amount of vitamin A that permitted resumption of growth. Ataxia was noted in one animal. Nervous running around the cage in circles appeared in some of the depleted animals. This was definitely reduced when sufficient vitamin A for growth was given. This was not cured by giving additional vitamin B complex in the form of brewers' yeast. Autopsy showed no evidence of otitis media or brain lesions. Skin lesions and corneal opacities appeared in a few of the animals. Dr. C. C. Higgins of The Cleveland Clinic autopsied one of the animals that had been depleted and then given a curative dose sufficient to produce a growth response. A calculus was found composed of calcium and magnesium phosphate with traces of calcium-carbonate and no oxalates or urates.

8339 C

## An Attempt to Formulate a Quantitative Theory of Membrane Permeability.

TORSTEN TEORELL.* (Introduced by W. J. V. Osterhout.)

*From the Laboratories of the Rockefeller Institute for Medical Research, New York City.*

The fundamental theories of electrolyte diffusion (Nernst,[1] Planck[2]) take into account the electrostatic forces that coöperate with the "osmotic" forces to cause the migration of ions. In these theories, however, no assumption has to be made as to the origin of the charge on the particles in the solution. Accordingly, we may expect that their predictions regarding ionic diffusion may be extended to include other cases of diffusion, where other charged elements are present, regardless of the constitution, shape, etc., of these elements.

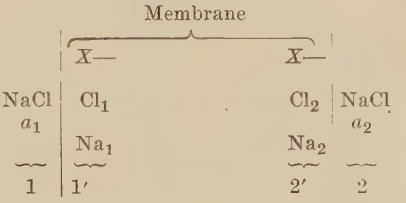
* Rockefeller Foundation Fellow.

[1] Nernst, W., *Z. physiol. Chem.*, 1888, **2**, 617; 1889, **4**, 154.

[2] Planck, M., *Wied. Ann.*, 1890, **40**, 561.

Diffusion of an electrolyte through a membrane may be such a case. The membrane may be regarded as having a charge due either to "adsorption," "dissociation," or "polar character," etc., but it is not necessary to make any further assumptions as to its nature. The effect of the membrane is regarded as that of an "added ion."

In order to demonstrate the usefulness of treating permeability problems as cases of simple diffusion we shall—for the sake of clarity, in a highly simplified way—try to show that the so-called "concentration effect" when NaCl diffuses across a "negative" membrane is theoretically predictable:




From one side of the membrane, NaCl, having the activity $a_1$, diffuses to the other side where the activity is $a_2$. The membrane may be represented as consisting of negative, immobile ions of the activity $X$, which is assumed to be constant throughout the membrane. In the steady state the ionic activities in the membrane surface layers may be $Na_1$, $Cl_1$ and $Na_2$, $Cl_2$. For electroneutrality it may be assumed that $Na_1 = (Cl_1 + X)$ and $Na_2 = (Cl_2 + X)$. Although the concentration of Na in the membrane differs from that of Cl the flux will be equal because the forces ("osmotic" plus electrical) operating on them are not equal. If the diffusion from 1′ to 2′ is sufficiently slow, the ionic distribution across 1-1′ and 2-2′ respectively approaches a thermodynamic equilibrium and we may write as an approximation

$$a_1^{-2} = Na_1 \cdot Cl_1 = Na_1 \cdot (Na_1 - X) \tag{1}$$

and

$$a_2^{-2} = Na_2 \cdot (Na_2 - X) \tag{2}$$

Evidently there are 2 "boundary" potentials present here between 1-1′ and 2-2′, which sum up to

$$\text{Total boundary potentials} = 58 \log [(a_1 \cdot Na_2) \div (a_2 \cdot Na_1)] \tag{3}$$

Besides these we have a "diffusion" potential between 1′-2′. As $X$ is constant along the distance in the diffusion layer, the "concen-

tration" gradient of Cl also is linear, because, as shown by Planck, the *total* concentration (Na) must always fall off linearly. Under such conditions the 1′-2′ potential can be most simply expressed by Henderson's formula,[3] which here reduces to

$$\text{Diffusion potential} = \frac{[(u - v) \div (u + v)]}{58 \log [\text{Na}_1(u + v) - X \cdot v] \div [\text{Na}_2(u + v) - X \cdot v]} \quad (4)$$

$u$ and $v$ are the constant "mobilities" (inverse friction coefficients) of Na and Cl. The total E.M.F. of the membrane is the sum of equations 3 and 4:

$$\text{Total E.M.F.} = 58 \left[ \log \frac{a_1 \cdot \text{Na}_2}{a_2 \cdot \text{Na}_1} + \frac{u - v}{u + v} \log \frac{\text{Na}_1 (u + v) - X \cdot v}{\text{Na}_2 (u + v) - X \cdot v} \right] \quad (5)$$

For calculations, $\text{Na}_1$ and $\text{Na}_2$ have to be expressed in terms of $a_1$ or $a_2$ and $X$, which is possible by means of equations 1 and 2.

If $X > 0$, it is found that the total E.M.F. is bound to vary with the absolute activities $a_1$ and $a_2$, even if the ratio $a_1 \div a_2$ is kept constant. This circumstance, however, is just what has been experimentally observed in a great many cases and has been called "concentration effect" (*cf.* Beutner,[4] Michaelis,[5] Osterhout[6]). Table I shows the numerical results of calculations according to equation 5 for this particular case.

TABLE I.

$X$ or membrane "activity" = 1. Membrane negative. Mobility relation $u : v$ in the membrane the same as in water. (Signs refer to the dilute solution in the external circuit.)

| $a_1$ | $a_2$ | Partial E.M.F. Boundary mv. | Partial E.M.F. Diffusion mv. | Total E.M.F. mv. |
|---|---|---|---|---|
| 100 | 10 | + 1.1 | —13.2 | —12.1 |
| 10 | 1 | +10.9 | —12.1 | — 1.3 |
| 5 | 0.5 | +20.5 | —12.0 | + 8.5 |
| 1 | 0.1 | +46.2 | — 5.4 | +41.8 |

Using NaCl the sign of the total E.M.F. will depend upon the relation between $X$ and the concentrations of the external solutions. In the comparable experiments of Beutner and of Michaelis, *et al.*, this relation seems to be such that only positive E.M.F. values are observed. These values, however, increase with decreasing con-

[3] Henderson, P., *Z. physik. Chem.*, 1907, **59**, 118.

[4] Beutner, R., Physical Chemistry of Living Tissues and Life Processes, Baltimore, Williams and Wilkins Co., 1933.

[5] Michaelis, L., *Kolloid-Z.*, 1933, **62**, 1. Michaelis, L., Ellsworth, R. McL., and Weech, A. A., *J. Gen. Physiol.*, 1926-27, **10**, 671.

[6] Osterhout, W. J. V., and Harris, E. S., *J. Gen. Physiol.*, 1928-29, **12**, 761.

centration, as predicted by the present theory, and the limit + 58 mv. is approached according to both experiments and calculations.

Further discussions of this and other permeability problems, regarded as cases of "forced" diffusion, will, it is hoped, be presented in other communications.

8340 C

## Apricot Seeds as a Source of Dehydrogenases.

CHARLES GURCHOT.

*From the Pharmacological Laboratory of the University of California Medical School, San Francisco.*

Increasing interest in various aspects of the mechanism of biological oxidation has developed a need for rich and reliable sources of dehydrogenases. An attempt was made to find such sources in appropriate plant material because of the possibility that such material might have an advantage in cheapness and in ease of handling. With the exception of yeasts, plants do not seem to have been much explored for dehydrogenases. Three samples of yeast from different manufacturers were found to be relatively poor sources for dehydrogenases, and samples from the same source varied greatly in such enzyme activity.

Preliminary search through representative available plant sources indicated the relative richness in dehydrogenases of the coatings of the seeds of various species of *Prunus*. With the skins removed, the seeds contain almost no dehydrogenases but are rich in lipases and emulsin. Such vegetables as beets and potatoes seem to be poor sources of dehydrogenases. Seeds of legumes and cereals are reported to be good sources of lactic dehydrogenase.[1] Representative assay data on dehydrogenase content are shown in Fig. 1 for extracts from beets, potatoes, yeast and seed coatings of almond, cherry, peach and apricot. Of these materials apricot seed coatings appear to be richest in dehydrogenases.

In extracting the dehydrogenases from fruit seeds, the seeds were first soaked for 24 hours in cold water to each liter of which one-half cc. of toluene was added. The skins were removed and extracted in a ball mill with disodium phosphate containing toluene, for 6 hours. To one liter of phosphate, 100 gm. of wet seed skins

[1] Anderson, B., *Z. Physiol. Chem.*, 1932, **210**, 15.

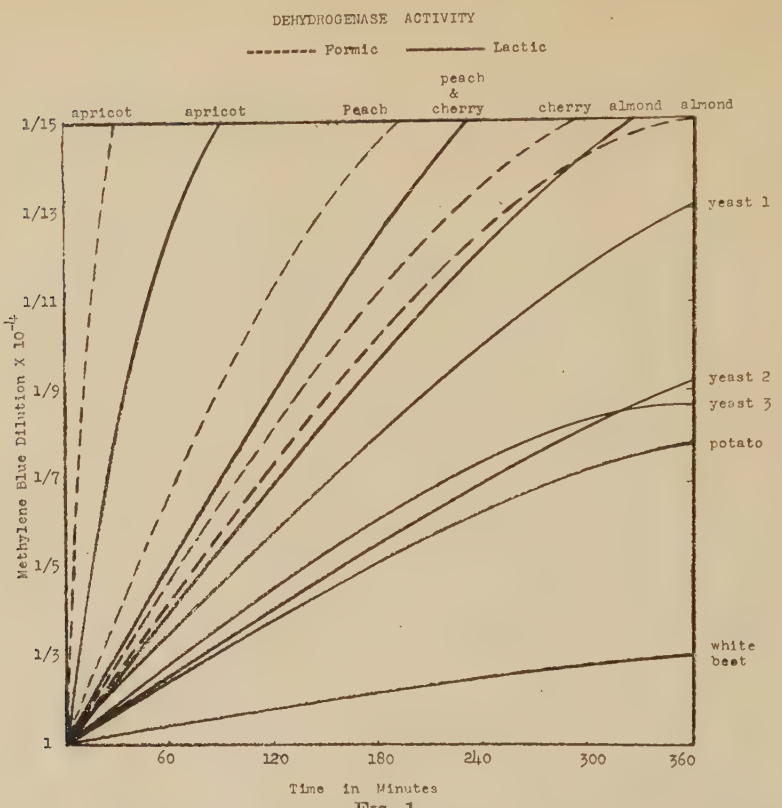



FIG. 1.
Dehydrogenase activity of various plant materials as determined by Thünberg methylene blue assay.

were added. The first extract contained most of the lactic and formic dehydrogenases. This was filtered and the skins extracted for another 6 hours with disodium phosphate. This second extract contained most of the malic, tartaric, and oxalic dehydrogenases. It was sometimes possible to separate lactic dehydrogenase from formic dehydrogenase by adsorbing the latter on alumina in acid buffer of pH 6.5. Formic dehydrogenase was then eluted with disodium phosphate. The phosphate filtrates were neutralized with dilute acetic acid and the enzyme precipitated with 5 volumes of 95% ethyl or methyl alcohol. The precipitate was centrifuged, washed with 95% alcohol, then ether, and dried in a vacuum desiccator over calcium chloride. The resulting material is gray and brittle. It can be purified to an almost white powder by dissolving in distilled water, dialyzing (to remove sulphydryl compounds which, when oxidized, compete with hydrogen acceptors and consequently

interfere with the methylene blue assay), and re-recipitating twice with alcohol.

Preparations so made were assayed by the Thünberg technique.[2] Tests were made in a total volume of 10 cc. of phosphate buffer of pH 7.6, containing 10 mg. of enzyme powder, 1 cc. of 1% sodium lactate or its molecular equivalent of another appropriate substrate and 1:10,000 methylene blue. This was placed in a water bath at 36°C. for 6 hours and compared during this period with a series of methylene blue standards ranging from 1:10,000 to 1:150,000 which is almost colorless. The time required for the color to pale to the next lower standard was recorded. By adopting a suitable standard one may adjust samples of varying activity as determined by assay to a uniform degree of effectiveness per unit quantity.

All enzymes tested were inactivated by heating for 15 minutes at 60°C. They were not inhibited by HCN. It is interesting to note that formic dehydrogenase was found to be the most active dehydrogenase tested in the seeds of the 4 species.

## 8341 C

### Effect of Cyclopropane on Isolated Intestinal Muscle.

S. A. PEOPLES* AND N. M. PHATAK. (Introduced by C. D. Leake.)

*From the Pharmacological Laboratory, University of California Medical School, San Francisco.*

It has been shown[1] that the *in vitro* effects of ether and ethylene on isolated intestinal muscle agree substantially with experimental evidence *in vivo*[2] and with clinical observations[3] that ether causes marked loss of intestinal tone and contractility while ethylene does not. It was also shown[1] that divinyl oxide, possessing a chemical structure combining characteristic features of both, is more like ethylene than ether in this respect as in other physiological actions. No reports so far seem to have been made regarding the effect of cyclopropane on intestinal muscle, although Waters and Schmidt[4]

2 Thünberg, T., *Quart. Rev. Biol.*, 1930, **5**, 318.

* Merck Fellow in Pharmacology.

1 Peoples, S. A., and Phatak, N. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **32**, 378.

2 Miller, G. H., *J. Pharm. Exp. Therap.*, 1926, **27**, 41.

3 Luckhardt, A. B., and Lewis, D., *J. Am. Med. Assn.*, 1923, **81**, 1851.

4 Waters, R. M., and Schmidt, E. R., *J. Am. Med. Assn.*, 1934, **103**, 975.

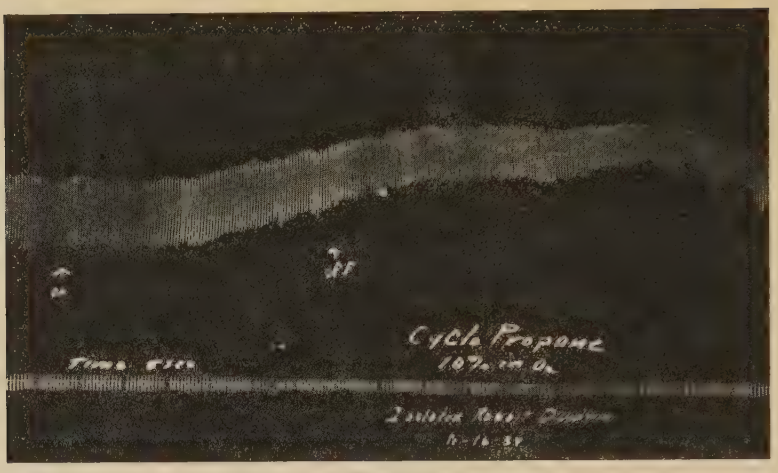



FIG. 1.
Effect of 10% cyclopropane in oxygen on intestinal muscle.

found slightly less post-operative intestinal distension after abdominal surgery with cyclopropane than with ether.

Segments of intestinal muscle about 2 cm. in length from the jejunum of a newly killed rabbit were suspended by the Magnus method from a muscle lever, in oxygenated Locke's solution at 37.5°C. Cyclopropane in concentrations in oxygen employed clin-


FIG. 2.
Effect of 25% cyclopropane in oxygen on intestinal muscle.

ically was bubbled through the solution in such a way as to bring it in equilibrium with the gaseous mixture as quickly as possible.

Thirty segments from 6 rabbits were tested for reactivity with acetylcholine and epinephrine in appropriate concentrations and washed carefully before applying the cyclopropane. In each case after bubbling into the bath concentrations of 10% to 25% cyclopropane in oxygen there was an increase in tone of the intestinal muscle with a decrease in amplitude of contraction (Figs. 1 and 2). Upon washing the cyclopropane from the bath there was a gradual return of the segment to its normal activity. The degree of gut stimulation was found to be roughly proportional to the concentration of cyclopropane up to 25% in oxygen at which maximum effect seemed to be obtained. In concentrations higher than this there was progressively less increase in tone. These observations indicate that less post-operative intestinal stasis and resulting distension should occur after the clinical use of cyclopropane than after ether.

## 8342 C

### Cumulative Toxicity of Emetine Hydrochloride in Guinea Pigs.

ELI A. ROSEN, R. R. MARTIN, AND NORMAN A. DAVID. (Introduced by C. D. Leake.)

*From the Department of Pharmacology, West Virginia University School of Medicine.*

The minimum lethal dose of emetine hydrochloride on subcutaneous injection of repeated small doses is practically the same as on single injection. Anderson and Leake[1] concluded from their own experiments and other observations reported in the literature that excretion of emetine after either oral or parenteral administration must be slow and also that little or no detoxication takes place in the tissues. Fischl and Schlossberger[2] summarize work indicating that the cumulative toxic effect of emetine hydrochloride may occur in many species. Nevertheless, there is some question as to the cumulative toxicity in the guinea pig in view of the single observation of Young and Tudhope[3] in which the total of divided doses of

1 Anderson, H. H., and Leake, C. D., *Am. J. Trop. Med.*, 1930, **10**, 249.

2 Fischl, V., and Schlossberger, H., *Handbook of Chemotherapy*, Baltimore, 1933.

3 Young, W. A., and Tudhope, G. R., *Trans. Roy. Soc. Trop. Med.*, 1927, **20**, 93.

emetine hydrochloride was equivalent to about 80 mg. per kg. before death occurred. Since little data is available on the cumulative toxicity of emetine when given in a series of graded doses, or on the toxicity in guinea pigs, the present work was designed to study both these points.

Seven groups of 10 guinea pigs each, ranging in weight from 150 to 250 gm. were used. Since it has been demonstrated that the $LD_{50}$ is approximately the same for many laboratory animals, Lake's[4] finding of a $LD_{50}$ of 20 mg. per kg. for white rats was arbitrarily chosen as a basis for the present work. Two groups of 10 guinea pigs each were given a single subcutaneous injection of this dose of emetine hydrochloride. The same dose of 20 mg. per kg. was administered to 4 similar groups of guinea pigs in divided doses of 1.0, 2.0, 4.0 and 10.0 mg. per kg., respectively, injected subcutaneously at intervals of 2 days until the full dose was given. As controls, 10 guinea pigs were given 20 subcutaneous injections of physiological saline.

The animals were weighed at frequent intervals during the 40-day period of observation. The usual picture of emetine intoxication was encountered, with marked lethargy, loss of weight and decreased appetite. Autopsy of animals dying revealed marked distension of the gut and general congestion of the viscera.

TABLE I.
Cumulative Toxicity of Emetine Hydrochloride in Guinea Pigs.

| No. of Doses | Mg./Kg. per Dose | Mortality Ratio No. Dying/No. Used | Av. Time of Death, Days from 1st Injection |
|---|---|---|---|
| 1 | 20 | 6/20 | 6.7 |
| 2 | 10 | 5/10 | 22.4 |
| 5 | 4 | 5/10 | 20.4 |
| 10 | 2 | 4/10 | 17.8 |
| 20 | 1 | 5/10 | 32.4 |
| 20 | saline | 0/10 | — |

From Table I it may seem that emetine hydrochloride shows a definite cumulative toxicity even when given in doses as small as one-twentieth of the MLD. While the small number of animals used impairs the significance of the results found, it is of interest to note that the cumulative toxicity of small divided doses was apparently greater than the acute toxicity when a single dose was administered.

*Summary.* The toxicity of emetine hydrochloride administered subcutaneously to guinea pigs is approximately the same whether the

[4] Lake, G. C., *U. S. P. H. S., Hyg. Lab. Bull.*, 1918, **113**, 2.

alkaloid is given in a single large dose or in repeated doses of 1/20 the acute MLD over a period of 40 days. Caution is indicated in the use of repeated doses in humans.

8343 P

## Different Potentialities of Male and Female Skin in Reeves Pheasants.*

C. H. DANFORTH.

*From the Department of Anatomy, Stanford University.*

The extraordinary similarity of feathers produced by male and female fowls after gonadectomy has led to the idea that the skin in the 2 sexes is potentially the same. This inference seems to be supported by the effects of endocrine injections and the results of skin transplantation. It is further strengthened by the fact that, although between different races there is much variation in response to endocrines, the 2 sexes of any one race react rather consistently. Nevertheless, there are frequently detectable, though generally slight, differences between feathers produced by birds that were originally male and those that were originally female. This suggests that the genotype of the male and that of the female may condition slightly different responses when all other factors are held constant.

A search for some form in which this problem could be approached more satisfactorily than in the common fowl revealed that the Reeves pheasant *(Syrmaticus reevesi)* is very satisfactory for the purpose. It is a species which breeds true to a standard type and has a plumage that is distinctive and varied, with none of the feathers of one sex duplicated by those of the other. The birds are rather expensive when purchased from dealers, but may be raised from eggs with relative ease.

Homoplastic skin transplantation immediately after hatching was chosen as the method affording the most delicate and dependable test. The procedure has the disadvantage of requiring a wait of several months between the beginning of an experiment and attainment of the final result. It also involves certain losses due to incompatibility of tissues of donor and host. But in many cases no incompatibility is revealed and the grafted skin appears to behave

* Investigation supported in part by the Rockefeller Fluid Research Fund of Stanford University.

normally in every respect, functioning as an integral part of the host. Plumage grown on such grafts provides a record of the reaction of feather follicles to the normal body fluids of the host, undisturbed by the shocks and readjustments accompanying gland removal or implantation and the fluctuations and uncertainties incident to hormonal injections. This advantage gives the method superiority over most other techniques available for the present study.

As yet the skin of only one region, the rump, has been adequately tested. Eleven specimens have been such as to yield pertinent data on the 4 types of donor-host combinations: male graft to male host, male to female, female to female and female to male. When donor and host were of the same sex, no deviation from the plumage normal to the sex was detected. It may be mentioned, however, that the females in this group were inadvertently lost before completely adult plumage had been attained. The combinations in which donor and host were of different sex resulted in 2 new types of feathers. There are consequently 4 different kinds of feathers that may be produced by skin of the rump in these pheasants. Their main features may be indicated briefly:

(1) If the skin has the genotype of the male and develops from the time of hatching in an environment of male hormones and tissue fluids, the feathers are marked by a black border, a broad orange zone and a black proximal area.

(2) If the genotype is male and the environment female, the feathers have a small terminal black spot or spangle which is surrounded and followed by a creamy area with slight olive green cast, which is followed in turn by an irregular dark band and a zone of brownish olive with a dark center.

(3) When both genotype and environment are female, the feathers have a dark olive brown or dull black center which comes to a point just proximal to the tip and is surrounded by a broad grayish border finely streaked and mottled with dull yellow.

(4) With genotype female and environment male, the feather is irregularly blotched with black, pale yellow and a little white.

Each of these types is quite distinct from the other 3, both in color and in markings. Data indicate that in this species the skin tissues are not 'equipotential', at least after hatching, inasmuch as in the production of feathers the follicles in the 2 sexes respond very differently to male and female hormonal complexes.

8344 C

## Cultivation of Ducrey Bacillus for Preparation of Vaccine.

GEORGE A. HUNT. (Introduced by S. H. Gray.)

*From the Snodgras Laboratory, City Hospital, Department of Public Welfare, City of St. Louis, Missouri.*

The preparation of Frei antigen for diagnosis of lymphogranuloma inguinale has emphasized the need of diagnostic procedures for ruling out the presence of chancroidal infection. Several culture methods have been described for determining the presence of active infection by the Ducrey bacillus,[1] but a skin sensitivity test (Dmelcos) is necessary for detecting previous or latent chancroidal infection. For the latter test it is desirable to obtain the Ducrey bacillus in pure culture and free from other organic matter.

Several methods have been described for the cultivation of the organism. Usually, a relatively large amount of pus from a "bubo" is added to blood agar, or blood media may be inoculated with a small amount of pus from the primary lesion. Teague and Deibert[2] described the use of clotted rabbit blood inactivated for a short time at 55°C., the organism being identified in pure or mixed cultures by the characteristic growth in chains of very small Gram-negative rods resembling streptococci.

The culture method of Teague and Deibert[2] is not suited for production of saline suspensions of the Ducrey organism, and subculture from blood or pus on the usual solid media was sparse or negative in our hands. Other workers have experienced similar difficulties.[3] Commercial preparations (saline suspensions of killed Ducrey bacillus) are available in Europe, but not generally obtainable in this country at the present time. Hence it was desirable to determine conditions which permit growth of the organism on solid media.

The following method gave satisfactory growth with all strains of Ducrey bacillus encountered in the laboratory of the City Hospital. It was found advantageous to employ tubes of clotted, inactivated blood as described by Teague and Deibert[2] for a preliminary culture medium, since such tubes were conveniently handled in the clinic. The tubes were inoculated with pus from chancroidal lesions and incubated for 24 to 48 hours at 37°C. Those tubes showing pure cultures or nearly pure cultures of Ducrey bacilli were selected for

1 Ducrey, A., *Monatch. f. prakt. Dermat.*, 1889, **9**, 387.

2 Teague, O., and Deibert, O., *J. Urol.*, 1920, **4**, 543.

3 DeWolf, H. F., and Van Cleve, J. V., *J. A. M. A.*, 1932, **99**, 1065.

use for subculture and isolation. A loopful of material from the blood tube was then spread on several blood agar slants (infusion agar as made by Wright,[4] 0.1% glucose and 3 to 5% whole blood), the wall of the test tube opposite the agar slant heated by passing through the Bunsen flame (usually 3 heatings of about one second each), the cotton stopper pushed into the tube and the tube tightly closed by a rubber stopper while the glass was still hot—a method of reducing the oxygen tension, described by Swartz[5] for the cultivation of gonococcus. The blood agar slants usually contained from 0.5 to 1.0 cc. of condensation water in the butt of the tube. Growth appeared promptly in most instances in the closed tube as typical colonies of tenacious consistency, with characteristic long chains of organisms in the water of condensation. Colonies transferred to new tubes grew well and were usually pure cultures. Suspensions were easily prepared by washing the growth from several slants, centrifugating and resuspending in saline. The growth was usually difficult to break into uniform suspensions, but this was accomplished by prolonged shaking, or agitation with a glass rod, or repeated aspiration in and out of a pipette with a narrow lumen at the tip.

The influence of reduced oxygen tension upon growth was marked in most instances. Blood agar slants similarly inoculated showed well developed colonies in the tubes that had been heated and stoppered, and no visible growth in heated but non-stoppered tubes. The growth of every strain was not enhanced to the same extent by this method, but all strains isolated showed some development in the closed tube in 48 hours. The strains isolated were not viable for long periods of time when stored at room temperature or in the ice-box. It was necessary to transfer cultures every 2 or 3 days.

Heat killed saline suspensions were prepared from 2 strains of the Ducrey bacillus and were tested by intracutaneous injections of the vaccines in patients with known chancroidal infection. These patients gave positive reactions with Dmelcos* vaccine,[6] negative reactions with Frei antigen; and Ducrey bacillus had been isolated from their primary lesions. Suspensions of both strains gave strongly positive skin reactions in these patients and no reactions in patients free from Ducrey infection.

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[4] Wright, H. D., *J. Path. and Bact.*, 1933, **37**, 257.

[5] Swartz, E. O., *J. Urol.*, 1920, **4**, 325.

*Dmelcos vaccine obtained through the kindness of May and Baker, Ltd., London.

[6] Stannus, H. S., A Sixth Venereal Disease, Wm. Wood & Co., Baltimore, 1933.

# New York Meeting

*New York Academy of Medicine, November 20, 1935.*

## 8345 P

### Serum Phosphatase Activity in Seventeen Cases of Osteoporosis Circumscripta of the Skull.

ALEXANDER B. GUTMAN, ETHEL BENEDICT GUTMAN, AND HAIG H. KASABACH. (Introduced by W. W. Palmer.)

*From the Department of Medicine and the Department of Radiology, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.*

The term osteoporosis circumscripta was introduced by Schüller[1] to describe a peculiar affection of the skull characterized radiographically by a marked decrease in density of large areas of the calvarium, the borders of the affected areas being irregular but sharply demarcated from the contiguous normal bone. The decrease in bone density in these cases is not due to bone destruction but rather to a localized osteoporosis, as post-mortem studies have shown.[2, 3, 4] It would appear that osteoporosis circumscripta of the skull, as defined roentgenographically, is identical with the abnormality of the skull described pathologically by Schmorl[5] as "hemorrhagic infarction of the bone."[6] The disease is quite distinct, however, and readily differentiated from xanthomatosis, cholesteatoma, multiple myeloma, metastatic neoplasm, and the like.

Osteoporosis circumscripta is of particular interest because, despite the dissimilarity in gross morphology, it appears to be closely related to Paget's disease. The majority of cases described thus far have also had typical Paget lesions in the skull or elsewhere in the skeleton. Where osteoporosis circumscripta was the only abnor-

1 Schüller, A., *Brit. J. Radiology*, 1926, **31**, 156.
2 Sosman, M. C., *Radiology*, 1927, **9**, 396.
3 Schellenberg, W., *Frank. Z. f. Path.*, 1931, **41**, 423.
4 Schüller, A., *Wien. klin. Wchenschr.*, 1931, **44**, 1577.
5 Schmorl, G., *Verh. deut. Path. Ges.*, 1930, **25**, 205.
6 Details in Kasabach, H. H., and Gutman, A. B., in preparation.

mality in the skull, follow-up studies in several instances have revealed the development of "cotton-wool" lesions typical of Paget's disease within 3 to 10 years. Most observers in the field have concluded that osteoporosis circumscripta is, in fact, a form of Paget's disease, probably an early, predominantly osteolytic phase initiating the more familiar stage of excessive bone formation.[2, 7, 8, 9]

Whether or not this is an over-simplified view of the matter, as might be inferred from Schmorl's observations,[5, 10] it seemed desirable to investigate the serum phosphatase activity in this disease. In polyostotic Paget's disease the serum phosphatase is consistently elevated, the increase being regarded by most investigators as a result of excessive bone formation.[11, 12, 13] Since in the related bone condition, osteoporosis circumscripta, over-production of bone does not take place, it was of interest to determine whether or not the serum phosphatase was elevated. No data relating to this subject could be found in the literature.

TABLE I.

Blood Analyses in 17 Cases of Osteoporosis Circumscripta of the Skull* (Associated with Typical Paget Lesions in 10 Instances).

| | | | Serum | | | |
|---|---|---|---|---|---|---|
| Age | Sex | Skeletal lesions associated with O.c of skull | Phosphatase Bodansky units per 100 cc. | Ca mg. % | Inorg. P mg. % | N.P.N. mg. % |
| 35 | ♂ | none | 1.6 | 8.9 | 4.2 | 52 |
| 60 | ♂ | " | 2.0 | 10.6 | 3.6 | 32 |
| 45 | ♀ | " | 4.5 | 11.0 | 3.4 | 30 |
| 38 | ♂ | " | 4.6 | — | 2.7 | — |
| 63 | ♂ | " | 5.4 | 9.7 | 2.6 | 29 |
| 31 | ♀ | osteofibroma(?) maxilla | 7.8 | 9.6 | 3.0 | — |
| 40 | ♂ | cyst tibia | 14.9 | 10.8 | 3.7 | 42 |
| 59 | ♂ | polyostotic Paget's d. | 23.0 | 10.8 | 3.6 | 29 |
| 48 | ♀ | " " " | 31.4 | 12.8 | 1.9 | 29 |
| 74 | ♀ | " " " | 32.1 | 10.5 | 3.2 | 32 |
| 64 | ♂ | " " " | 32.5 | 11.5 | 3.2 | 31 |
| 54 | ♀ | " " " | 34.6 | 10.2 | 3.8 | 32 |
| 58 | ♀ | " " " | 36.5 | 10.1 | 3.8 | 36 |
| 70 | ♀ | " " " | 40.1 | — | 3.8 | 44 |
| 43 | ♂ | " " " | 45.7 | 10.8 | 3.5 | — |
| 36 | ♂ | " " " | 52.5 | 10.2 | 3.2 | 31 |
| 37 | ♀ | " " " | 57.9 | 9.8 | 3.6 | 25 |

* Six reported by Kasabach and Dyke[9]; the other to be published by Kasabach and Gutman.[6]

[7] Weiss, K., *Fort. a. d. Geb. Roentgenstr.*, 1930, **42**, 376.

[8] Meyer-Borstel, H., *Fort. a. d. Geb. Roentgenstr.*, 1930, **42**, 589.

[9] Kasabach, H. H., and Dyke, C. G., *Am. J. Roentgen.*, 1932, **28**, 192.

[10] Schmorl, G., *Virchow's Arch. f. Path. Anat.*, 1932, **283**, 694.

[11] Kay, H. D., *Physiol. Rev.*, 1932, **12**, 384.

[12] Bodansky, A., and Jaffe, H. L., *Arch. Int. Med.*, 1934, **54**, 88.

[13] Gutman, A. B., and Kasabach, H. H., *Am. J. Med. Sci.*, 1935, in press.

Our results (Table I) indicate that in uncomplicated osteoporosis circumscripta of the skull the serum phosphatase activity is within normal limits or is only slightly increased (cases 1-5). When associated with typical Paget involvement of the skeleton, however, considerable increases in serum phosphatase activity occur (cases 8-17), of the same order as those seen in Paget's disease not associated with osteoporosis circumscripta. Our results are in accord with, and may be regarded as further substantiation of the view that increased phosphatase activity of the blood in bone disease is an expression of an increase in cellular processes leading to bone formation.[14]

Serum phosphatase was determined by A. Bodansky's method;[15] serum calcium by Clark and Collip's modification[16] of the Kramer and Tisdall method; serum inorganic phosphorus by the Kuttner-Lichtenstein method,[17] with corrections for deviations from Beer's law.[18]

## 8346 P

## Distribution of Blood Groups and Agglutinogen M Among Indian "Blackfeet" and "Blood" Tribes.*

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The early work on the distribution of blood groups among the various races led to the hope that results would indicate clear-cut racial relationships, but the significance of this line of investigation has been overestimated.[1] This was the conclusion of Grove,[2] who

14 Robison, R., The Significance of Phosphoric Esters in Metabolism, New York University Press, New York, 1932.

15 Bodansky, A., *J. Biol. Chem.*, 1933, **101**, 93.

16 Clark, E. P., and Collip, J. B., *J. Biol. Chem.*, 1925, **63**, 461.

17 Kuttner, T., and Lichtenstein, L., *J. Biol. Chem.*, 1930, **86**, 671.

18 Bodansky, A., *J. Biol. Chem.*, 1932, **99**, 197.

* The authors are indebted to the following for help in obtaining the material: Rev. S. H. Middleton and Miss Megarry of the St. Paul's School, Dr. J. K. Mulloy, Senior Physician at the Blood Reservation, and Miss G. D. Knapp of the Blackfeet Agency.

1 Wiener, A. S., Blood Groups and Blood Transfusion, 1935, C. C. Thomas, Springfield, Ill.

2 Grove, E. F., *J. Immunol.*, 1926, **12**, 251.

in her studies on various aborigines found branches of the same race showing widely different incidences of the 4 blood groups. Previous reports by Coca and Deibert[3] and Snyder[4] supported the view that American Indians are characterized by a high percentage of group O, but more recently Matson and Schrader[5] described other tribes of Indians ("Blackfeet" of Montana and "Blood" of Canada) with a very high incidence of group A. Shanklin[6] found a high percentage of group O among certain Arabian tribes, others having high values for A and B.

All these studies, however, were limited to one set of factors—the 4 blood groups. Other agglutinable blood properties, the factors M, N, and P and the subgroups $A_1$ and $A_2$, and the peculiar taste reaction to para-ethoxy-phenyl-thio-urea, were shown to exhibit characteristic racial differences[7, 8, 9] and obviously future racial investigation of all these properties may yield more significant data.

The present report deals with a study of the incidence of the blood groups and the factor M in pure-blooded "Blackfeet" and "Blood" tribes. The results show that while these Indians differ radically from the Kansas Indians in the distribution of the 4 blood groups, they behave alike in having a very low incidence of M negative reactions, in comparison with the incidence among white individuals. The contrasting figures for the M reactions among the "Blackfeet" and "Blood" Indians on the one hand, and among white individuals of Montana on the other, were obtained with the use of the same anti-M serum.

These tests also serve to confirm the original observations of Matson and Schrader on the high incidence of the A factor in the "Blackfeet" and "Blood" tribes.

Observations were made also on the frequency of taste-blindness to para-ethoxy-phenyl-thio-urea in these Indians and, in harmony with the M results, these tests show that the incidence of non-tasters distinctly approximates the low figure (6%) previously found by Levine and Anderson in the Kansas Indians, rather than that found in tests made simultaneously on white individuals of Montana (28%). Accurate figures cannot be given at this time on account

3 Coca, A. F., and Deibert, O., *J. Immunol.*, 1923, **8**, 487.

4 Snyder, L. M., *Am. J. Phys. Anthrop.*, 1926, **9**, 233.

5 Matson, G. A., and Schrader, H. F., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1380; *J. Immunol.*, 1933, **25**, 155.

6 Shanklin, Wm., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 754.

7 Landsteiner, K., and Levine, Ph., *J. Immunol.*, 1929, **16**, 123.

8 Landsteiner, K., and Levine, Ph., *J. Immunol.*, 1930, **18**, 87.

9 Levine, Ph., and Anderson, A. S., *Science*, 1932, **75**, 497.

TABLE I.

| | Group | | | | M | |
|---|---|---|---|---|---|---|
| | 0 | A | B | AB | + | — |
| Pure Blackfeet and | 36 | 138 | 0 | 2 | 149 | 3 |
| Blood Indians (this study) | 20.5 | 78.4 | 0 | 1.1 | 98 | 2 |
| Pure Kansas Indians | 156 | 49 | 0 | 0 | 195 | 10 |
| (Landsteiner and Levine[7]) | 76.1 | 23.9 | | | 95.1 | 4.9 |
| White, Montana | 84 | 59 | 26 | 6 | 130 | 43 |
| (this study) | 48 | 34 | 15 | 3 | 75 | 25 |
| White, New York | 758 | 648 | 226 | 76 | 1382 | 326 |
| (Landsteiner and Levine[7]) | 44.4 | 37.9 | 13.2 | 4.5 | 80.9 | 19.1 |

The first line of figures indicates absolute numbers examined; the figures below give the corresponding percentages.

Of the "Blackfeet" Indians, 24 were in Group O, 82 in Group A, and 1 in AB; of these only 2 were M negative. Of the "Blood" tribes, 12 were in Group O, 56 in Group A, and 1 in AB. M tests were made on 45 of these specimens and only one was negative.

of difficulty of interpreting certain taste reactions, as for instance, those reported as sweet.

## 8347

### Susceptibility to Lysozyme of Staphylococci.

RICHARD THOMPSON AND DEVORAH KHORAZO. (Introduced by C. W. Jungeblut.)

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In his review on lysozyme Fleming[1] has stressed the fact that the extreme susceptibility of many air saprophytes has resulted in a general impression that pathogenic organisms are not affected by this enzyme. He cites his own and other work to prove that pathogenic organisms are attacked if the concentration of lysozyme is great enough. Ridley[2] reported that the ability to resist lysozyme seemed to determine pathogenicity. Regarding staphylococci, Neisser[3] quotes Nakmura and also Kopp as presenting evidence that susceptibility to lysozyme is to be regarded as indicating that a staph-

1 Fleming, A., *Proc. Roy. Soc. Med.*, 1932, **26**, 71.

2 Ridley, F., *Proc. Roy. Soc. Med.*, 1928, **21**, 1495.

3 Neisser, M., *Handbuch der path. Microorg.*, Kolle, Kraus and Uhlenuth, Berlin, 1927, **4**, 437.

ylococcus is not a "Pyococcus", *i. e.*, of the pathogenic type. Cavka and Prica[4] found that tears acted only upon air staphylococci and not upon those isolated from lesions.

In experiments with a number of strains of staphylococci and sarcinae, we have observed a certain relationship between type of pigment, mannite fermentation and coagulase production on the one hand and susceptibility to lysis by lysozyme on the other, which we believe justifies a brief report. Since the work of Gordon[5] and Hine[6] it has been generally accepted that mannite-fermenting staphylococci are more likely to be pathogenic than those which do not have this property. Much,[7] Gross[8] and others have shown that pathogenic staphylococci have the power of coagulating oxalated human plasma. This action has been ascribed to an enzyme termed coagulase. The study of the cultures reported here has been limited to the above mentioned properties. Hemolysin, skin toxin, leucocidin or the production of specific antigen have not been studied.

*Lysozyme Test.* Saline suspensions of 18-24-hour agar-slant cultures of the various organisms were made to produce a turbidity equivalent to a No. 8 barium sulphate standard. 0.5 cc. amounts of progressive saline dilutions of fresh egg-white or of purified,* concentrated lysozyme from egg-white were added to equal quantities of the bacterial suspensions in small test tubes. The tubes were incubated in a water bath at 37°C. for 24 hours and readings made by comparing the turbidities with that of a control tube of organisms plus saline. The efficacy of the lysozyme preparation was controlled by titration with a sarcina of known susceptibility.

*Mannite Fermentation.* Cultures were inoculated into tubes of 1% Difco mannite in beef infusion broth. After 3 days' incubation the presence of acid was tested for by adding 2 drops of bromcresol purple.

*Coagulase.* Oxalated plasma was obtained by placing 10 cc. of fresh human blood into a centrifuge tube containing 0.02 gm. of potassium oxalate, thoroughly mixing and then centrifuging out the cells. 0.3 cc. of the plasma was mixed with 0.2 cc. of an 18-24-hour

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4 Cavka, V., and Prica, M., *Arch. f. Ophth.*, 1929, **121**, 740.

5 Gordon, M. H., Rep. (34th) Med. Off. Local Govt. Bd., 1906, 387.

6 Hine, T. G. M., *Lancet*, 1922, **2**, 1380.

7 Much, H., *Biochem. Z.*, 1908, **14**, 143.

8 Gross, H., *Cent. Bact. I Orig.*, 1931, **122**, 354.

*Methods of purification described elsewhere.[9]

9 Meyer, K., Palmer, J. W., Thompson, R., and Khorazo, D., *J. Biol. Chem.*, in press.

broth culture of the organism being tested. Partial or complete clotting after 2 hours' incubation at 37°C. indicated the production of coagulase.

*Pigment.* The pigment was classified as orange, lemon or white by the appearance of the massed growth from a 72-hour agar plate placed upon white filter paper.

One hundred and twenty strains of staphylococci and sarcinae from various sources, chiefly normal and inflamed conjunctivae were tested by these methods. The results are summarized in Table I.

TABLE I.

| Classified by | Total No. of strains | No. of strains dissolved by lysozyme | % of strains dissolved by lysozyme |
|---|---|---|---|
| | Staphylococci | | |
| Pigment | | | |
| Orange | 60 | 10 | 16 |
| White | 39 | 19 | 48 |
| Lemon | 4 | 4 | 100 |
| Mannite Fermentation | | | |
| Positive | 54 | 4 | 8 |
| Negative | 49 | 29 | 59 |
| Coagulase Production | | | |
| Positive | 64 | 7 | 10 |
| Negative | 39 | 22 | 56 |
| | Sarcinæ | | |
| Pigment | | | |
| White | 5 | 5 | 100 |
| Lemon | 11 | 11 | |
| Rose | 1 | 1 | |

None of the sarcinae or the lemon staphylococci fermented mannite or produced coagulase. Seventy-eight percent of the orange strains and 18% of the white strains fermented mannite. Eighty-three percent of the orange strains and 36% of the white strains produced coagulase.

For simplification the various degrees of susceptibility to lysozyme have not been shown. The lemon staphylococci and all the sarcinae showed a sensitivity to lysozyme approaching or as great as that of the test organism. The white and orange staphylococci in no case showed marked susceptibility. The suspensions of these were seldom completely dissolved and in most cases only partial clearing occurred in a lysozyme dilution of 1-4 or 1-8.

*Summary.* All strains of sarcinae (17) and lemon staphylococci (4) studied were readily dissolved by lysozyme. None of the 99 white or orange staphylococci studied showed marked susceptibility, but certain strains were dissolved by higher concentrations of enzyme. There was a definite, although not absolute, negative corre-

lation between orange pigment, the ability to ferment mannite or produce coagulase on the one hand and susceptibility to lysozyme on the other.

8348 P

## Quantitative Use of Neufeld Reaction with Special Reference to Titration of Type II Antipneumococcic Horse Sera.

FRANCES L. CLAPP, SARA W. PHILLIPS AND HELENE J. STAHL.
(Introduced by A. F. Coca.)

*From the Lederle Laboratories, Inc., Pearl River, New York.*

The Neufeld reaction has recently been advocated[1] for the diagnosis of the different types of pneumonia directly from the patient's sputum. For this purpose, he advised a monovalent antipneumococcic rabbit serum in order to avoid the non-specific reactions which he obtained with the use of antipneumococcic horse serum. More recently, Cooper and Walter[2] reported this same "swelling" phenomenon when antipneumococcic serum from rabbits was mixed with the homologous pure culture grown in artificial media. This observation led us to examine different samples of antipneumococcic serum by mixing them with specific pure cultures and observing the degree of capsular swelling which resulted. It was found that a serum high in mouse protective units caused much more swelling than a serum low in mouse protective units, also that a serum of high potency could be diluted many times and still give a typical "swelling" reaction. These experiments suggested a possible quantitative application of the Neufeld reaction.

Preliminary work with Type II broth cultures and specific antipneumococcus horse sera showed a definite linear relationship between the number of organisms used and the least amount of antibody required to produce an enlarged capsule. This reaction appeared to be specific. The following method of titration of Type II antibody was then developed:

The culture, either live or formalinized, is diluted with 1% peptone to the required density; the serum is diluted with physiological saline solution. Appropriate amounts of a constant culture dilution and of varying serum dilutions are measured with standard platinum loops, placed on a thin cover-slip, mixed well with a loopful of

[1] Sabin, *J. A. M. A.*, 1933, **100**, 1584.

[2] Cooper and Walter, *Am. J. Pub. Health*, 1935, **25**, 469.

methylene blue solution, and inverted on a microscope slide. These preparations are incubated 30 minutes in a moist chamber at 37°C., and then examined under a fluorite oil-immersion lens. The end-point is taken as the highest dilution of serum producing definite capsular swelling. A control serum of known unit value is used as a basis for comparison and for calculation of potencies of the unknown sera.

TABLE I.
Comparative Values Obtained in the Measurement of Type II Antibody in 53 Samples of Antipneumococcic Horse Sera.

| Horse No. | Capsular-Swelling Units | Mouse Units | Horse No. | Capsular-Swelling Units | Mouse Units |
|---|---|---|---|---|---|
| 4432 | 400 | 600 | 6490 | 150 | 200 |
| 4479 | 175 | 225 | 6491 | 300 | 250 |
| 4616 | 200 | 175 | 6494 | 45 | 50 |
| 5373 | 100 | 25 | 6498 | 75 | 50 |
| 6071 | 85 | 100 | 6499 | 50 | 75 |
| 6222 | 75 | 75 | 6515 | 75 | 75 |
| 6225 | 85 | 50 | 6625 | 25 | 35 |
| 6229 | 50 | 35 | 6626 | 75 | 75 |
| 6309 | 100 | 10 | 6627 | 75 | 75 |
| 6313 | 200 | 175 | 6628 | 25 | 15 |
| 6325 | 150 | 75 | 6629 | 25 | 35 |
| 6333 | 100 | 75 | 6630 | 275 | 300 |
| 6335 | 150 | 100 | 6631 | 25 | 35 |
| 6336 | 225 | 300 | 6634 | 35 | 25 |
| 6369 | 200 | 200 | 6635 | 50 | 50 |
| 6441 | 125 | 50 | 6639 | 75 | 50 |
| 6450 | 60 | 75 | 6642 | 65 | 25 |
| 6451 | 65 | 35 | 6649 | 5 | 2 |
| 6454 | 50 | 50 | 6650 | 175 | 35 |
| 6459 | 175 | 100 | 6653 | 125 | 100 |
| 6461 | 50 | 35 | 6654 | 60 | 25 |
| 6463 | 65 | 10 | 6774 | 75 | 15 |
| 6464 | 10 | 5 | 6775 | 50 | 25 |
| 6466 | 75 | 50 | 6776 | 65 | 10 |
| 6478 | 75 | 50 | 6777 | 25 | 10 |
| 6479 | 125 | 150 | 6831 | 50 | 5 |
| 6481 | 110 | 150 | | | |

This technic was applied to 53 samples of Type II antipneumococcic horse sera which were also assayed by the mouse protection test. The 2 sets of unit values thus obtained were correlated according to the Pearson formula. The coefficient of correlation between the values obtained by the use of the Neufeld reaction and by the mouse protection test was found to be 0.89.

Similarly, 20 samples of Type II antipneumococcic horse sera were tested by the method described and by Felton's equivalent-combining method.[3] The coefficient of correlation obtained from these results by the Pearson formula was 0.91.

[3] Paper read before the American Association of Immunologists, New York City, April 17, 1935.

The method reported with slight variations in technic has been used with similar success in the titration of Type I antipneumococcic horse sera. A limited number of tests with concentrated antibody solutions have given promising results. No prozones have been encountered. Preliminary experiments indicate that the test may be applied to other types of antipneumococcic sera.

Since a positive reaction may be demonstrated with a solution containing less than 5 units of antibody per cc., and since the technic described requires only a small amount of serum, possible clinical applications such as the demonstrations of specific antibody in human sera are suggested.

We have been able to demonstrate capsular swelling in mixtures of Type I antimeningococcic serum with homologous organisms. This reaction suggests many possible applications, such as the diagnosis of the type of meningitis directly from the spinal fluid. It also indicates that with appropriate sera, it may be possible to demonstrate capsular-swelling reactions with other kinds of bacteria.

8349 P

## Polarity in Lethal Action of Electric Current.

VICTOR SCHECHTER. (Introduced by A. J. Goldforb.)

*From the College of the City of New York.*

Electric currents have been shown to be capable of directing or determining the growth of certain organisms of simple symmetry.[1,2,3] The present is a report of experiments in which the application of direct electric current resulted in partial death of the organism; the effect being localized in a polar fashion. The results given below were obtained with the Bryophyte *Conocephalus*. Lunularia and Marchantia reacted similarly but not as definitely.

The plants were cleaned. Most of the rhizoids and the apex and base were clipped off. Current was applied through an agar bed (usually containing nutrients) upon which the plants were placed in intimate contact. It was found that death occurred in that part of the plant which lay toward the anode. This effect was not due to the creeping of toxic materials from the electrodes as suitable pre-

[1] Barth, L. G., *Physiol. Zool.*, 1934, **7**, 340.

[2] Lund, E. J., *J. Exp. Zool.*, 1924, **39**, 357.

[3] Schechter, Victor, *J. Gen. Physiol.*, 1934, **18**, 1.

cautions were taken. Also, successive rows throughout the experimental dish were similarly affected while the region toward the cathode remained alive in all plants.

The extent of the dead region increased with greater current density; also with greater duration of exposure. Larger pieces were more susceptible than small, *i. e.*, the percentage dead region/plant size was greater. No difference was detected in quantity of effect between plants oriented in opposite directions; that is, apex and base were equally susceptible when turned toward the anode. Regeneration did not, of course, occur toward the anode, but was not absent elsewhere. As a result new growths were more frequently seen on plants turned with apex toward the cathode.

This report is based on 116 plants which were subjected to direct currents of approximately ½ to 6δ for a period of time up to 350 hours. The dead region comprised 5 to 63% of the length of the thallus. Large plants (about 6 cm. long) showed about 20% more injury than small ones (1 cm. or less) for the same duration and density of current.

Perhaps some light may be thrown by these findings on those cases cited[1, 2, 3] in which the polarity of growth is affected by electricity. (It is not intended to infer, however, that toxic action at one pole is necessarily responsible there.)

8350 C

## Evidence for the Presence of a Diffusible Organic Substance in Blood Which Accelerates Blood Clotting.

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Dialysis of blood plasma, it has been presumed, removes only one component essential to the blood clotting mechanism, namely, calcium. However, during the course of certain experiments in which plasma was very thoroughly dialyzed, the authors observed that when the plasma proteins were redissolved in a saline solution containing an adequate amount of calcium, no clot was formed for 24 or 48 hours. If to this artificial plasma there is added a small portion of the ultrafiltrate of whole blood or serum, the formation of a firm clot is induced within an hour or less.

This observation seems to indicate the presence of a dialyzable substance in the blood other than calcium ion which is concerned in the blood clotting process. The substance appears to be an organic compound since the power of accelerating blood clotting is lost if the serum ultrafiltrates are charred.

Either citrated or oxalated plasma may be used to demonstrate this phenomenon. However, a thorough dialysis of the plasma is required, ordinary dialysis against tap water not being sufficient. In the present experiments the dialysis was carried out in a rocking dialyzer[1] at the temperature of 5° against running distilled water. After about 2 to 3 days of dialysis, an artificial plasma is prepared by adding sodium chloride, sodium bicarbonate, sodium phosphate, magnesium chloride, and glucose in the amounts required to produce the concentration found in blood. The pH is adjusted to 7.4 by bubbling in $CO_2$. Calcium is not added until it is desired to initiate a clotting experiment. The plasma proteins disperse completely in the above menstrum, giving a solution which is no more turbid than the original plasma.

If properly dialyzed, such an artificial plasma shows no sign of clotting for 24 hours or longer after calcium has been added. However, on the addition of serum ultrafiltrate, a firm clot is formed in from 30 minutes to 1 hour. The amount of ultrafiltrate required is small, as little as 0.1 cc. in 5 cc. of artificial plasma being sufficient to reduce the clotting time to about 2 hours. Less than this proportion gives indefinite results.

This phenomenon is not limited to any particular animal species. It was demonstrated by the authors on plasma from human, dog, sheep, beef, and chicken blood. Neither is the substance species specific since an ultrafiltrate from dog serum will accelerate the clotting of dialyzed human plasma and vice versa.

The results of tests to characterize the clot accelerating substance in the ultrafiltrate are given in Table I.

TABLE I.

Experiments on the Accelerating Effect of Serum Ultrafiltrates on Blood Clotting.

| Experimental Procedure | Clotting Produced |
|---|---|
| Addition of untreated serum ultrafiltrates | + |
| After boiling ultrafiltrate for 45 minutes | + |
| " charring ultrafiltrate | — |
| " adsorption with permutit | + |
| " extensive bacterial contamination | + |

+ indicates acceleration of clotting.
— indicates no acceleration of clotting.

[1] Kunitz, M., and Simms, H. S., *J. Gen. Physiol.*, 1928, **11**, 641.

From the table it is seen that of the procedures tried, only heating of the ultrafiltrate to charring destroyed the accelerating effect on the clotting process.

The substances known to influence blood clotting which might be removed by dialysis are sulfhydryl compounds[2] and perhaps cephalin, the latter possibly by adsorption on the walls of the dialyzing membrane. Tests made to determine if these materials were in any way concerned gave negative results. Addition of sulfhydryl compounds in the form of cysteine and thioglycollic acid and of an ether extract of brain to supply cephalin did not produce a reduction in the clotting time of the artificial plasma.

## 8351 C

### Fibrinolytic Titer of Scarlatinal Streptococci.*

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*From the Connaught Laboratories, University of Toronto, and the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.*

Applying the Tillett-Garner plasma-clot technic[1] with rate of thrombolysis as the quantitative index, Dack, Woolpert and Hoyne[2] found a slight correlation between thrombolytic titer and clinical severity of scarlet fever. At the time of the publication of their paper a similar study of scarlatinal streptococci was in progress in our laboratories We had selected the more delicate isolated fibrin-clot technic, however, with serial-dilution methods of determining lytic titer.

The 60 strains of *S. scarlatinae* used in this study were originally isolated in The Connaught Laboratories, University of Toronto. With 43 of these strains Dick-toxin titrations have been made by one of us (F). The fibrinolytic titrations were made in Stanford University (M). Both series of titrations are summarized in Table I.

From Table I it is seen that there is a slight correlation between toxin-titer and fibrinolysin-titer with scarlatinal streptococci. The

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[2] Carr, L. J., and Foote, F. S., *Arch. Surg.*, 1934, **29**, 227.

* Work supported in part by the Eli Lilly and Co. Streptococcus Research Fellowship of Stanford University and in part by the Rockefeller Fluid Research Fund of Stanford Medical School.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Dack, G. M., Woolpert, O. C., and Hoyne, A. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1431.

TABLE I.

Correlation Between Toxin-titer and Fibrinolysin-titer.

Each fibrinolytic titration was made in triplicate, using 3 different sub-cultures and 3 different samples of presumably normal human fibrin, quantities, dilutions, temperatures, etc., being identical with those used by Tillett and Garner.[1] The maximum serial dilution of a 24-hour broth culture giving complete liquefaction of the serum-free human fibrin-clot, by the end of 2 hours, was assumed to contain one lytic unit.

The table records average titers, expressed in terms of the nearest routine serial dilution. Thus, all strains giving average fibrinolytic titers between 200 to 300 units per cc. are grouped under the routine titer 256 units. Strains giving toxin-titers, between 3000 to 6000 units, are grouped under the routine titer 4000 units (4M).

In order to determine the degree of correlation, the total population was divided into 4 approximately equal population quadrants (see lines). The upper left-hand quadrant (*TF*) is composed of strains simultaneously superior in both fibrinolysin (*F*) and toxin (*T*) production. The lower right-hand quadrant (*tf*) is composed of strains simultaneously inferior in both these functions. The 2 other quadrants consist of strains with one superior or one inferior function. A rough percentage of correlation may be calculated from the equation:

$$C = (TF + tf)/Population.$$

| Fibrinolytic units per cc. | No. of strains yielding the following number of toxin-units per cc. | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 32M | 16M | 8M | 4M | 2M | M | 500 | 250 | <125 |
| 1024 | — | — | — | — | — | — | — | — | — |
| 512 | 1 | 2 | 3 | — | — | — | — | — | 1 |
| 256 | 1 | — | 5 | — | 1 | — | — | — | 1 |
| 128 | — | — | 2 | 3 | — | — | 1 | — | — |
| 64 | 1 | — | 1 | — | 1 | — | — | 1 | — |
| 32 | 1 | 1 | — | — | 1 | — | 1 | — | — |
| 16 | — | — | 1 | — | — | 1 | 2 | — | — |
| 8 | — | — | — | — | — | 1 | 1 | — | — |
| <4 | — | 1 | 1 | — | — | 2 | 1 | 1 | 2 |

*TF = 17 strains. tf = 15 strains.* $(17 + 15)/43 = 75\%$

2 population quadrants composed of strains either simultaneously superior, or simultaneously inferior in both fibrinolysin and toxin production, together contain 32 strains, or 75% of the population.

TABLE II.

Correlation Between Toxin-titer and Symptomatology.

Classification of the cases as "mild," "moderate," or "severe" was based on the temperature, duration of illness and number of complications. Correlation percentage calculated as in Table I by dividing the total population into four population groups (see lines).

| Symptomatology | No. of strains yielding the following number of toxin units per cc. | | | | | | | | | Horizontal group-ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | 32M | 16M | 8M | 4M | 2M | M | 500 | 250 | <125 | |
| Severe | 1 | 1 | 9 | 2 | 1 | 1 | 1 | — | 1 | *13:4* |
| Moderate | — | — | — | — | — | 1 | 2 | — | — | |
| Mild | 3 | 3 | 4 | 1 | 1 | 2 | 3 | 2 | 2 | *11:13* |
| Vertical group-ratio | | | *13:11* | | | *4:13* | | | | $(13 + 13)/41 = 63\%$ |

An attempted correlation of toxin-titer and symptomatology is recorded in Table II.

From Table II it is seen that there are 13 strains in the left-upper quadrant in which high toxin-titer is associated with "severe" symptoms, and 13 strains in the right-lower quadrant in which low toxin-titer is associated with "mild" or "moderate" symptoms. These 2 quadrants together contain 26 strains or 63% of the population.

An attempted correlation of fibrinolysin-titer with symptomatology is recorded in Table III.

TABLE III.
Correlation Between Fibrinolysin-titer and Symptomatology.

| Symptomatology | No. of strains yielding the following number of fibrinolytic units per cc. | | | | | | | | | Horizontal group-ratio |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1024 | 512 | 256 | 128 | 64 | 32 | 16 | 8 | <4 | |
| Severe | — | 4 | 9 | 7 | 1 | — | — | — | 1 | *20:2* |
| Moderate | — | 2 | — | — | 1 | 2 | 2 | — | 1 | |
| Mild | — | 3 | 3 | 1 | 3 | 4 | 3 | 2 | 6 | *9:24* |
| Vertical group-ratio | | | *20:9* | | | *2:24* | | | *(20 + 24)/55* = **80%** | |

Table III shows an 80% correlation between fibrinolysin-titer and symptomatology, as contrasted with the 63% correlation between toxin-titer and symptomatology.

Since practically all of the "severe" cases in this series had one or more complications, and since the great majority of the milder cases were uncomplicated, the 2 relative percentages (80%:63%) confirm the conclusion of Dack and his coworkers, that high fibrinolysin-titer plays a demonstrable rôle in determining complications in scarlet fever.

8352 P

## Gonadotropic Substance in the Blood of Normal Humans.

S. C. FREED. (Introduced by Samuel Soskin.)

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It has been shown that, by utilizing the synergistic action of certain pituitary extracts, otherwise unappreciable amounts of a "prolan-like" gonadotropic substance may easily be demonstrated in adult male urine concentrates[1] and in the urine of male and female

children.[2] We have extended such observations to the blood of normal humans of both sexes and at varying ages.

The method employed was similar to that used in our previous work on urines.[2] The ammoniated alcohol extract of sheep pituitary which we used, when injected into 25-day-old rats over a 3-day period, resulted in ovaries weighing about 24 mg. at the end of 96 hours. The uteri were not affected. The injection of 1 cc. of human blood serum daily, for 3 days, caused no change in the ovaries or uteri of similar rats at the end of the same time interval.

When the above injections were combined, surprisingly great changes were observed. The blood sera of 34 individuals, both male and female, and ranging in age from 2-66 years, invariably caused augmentation of ovarian weight. These weights reached as high as 140 mg., the average being about 80 mg. (200-600% augmentation). The ovaries were heavily luteinized. In addition, the vagina was open in most cases and the uterus was considerably enlarged, weighing between 65-80 mg. The uteri were not distended with fluid.

When synergized by a suitable pituitary extract, the injection of 1 cc. of human blood serum per day for 3 days has been shown to produce an augmentation in the weight of the 25-day-old rat's ovaries equivalent to that produced by 5-10 rat units of A-PL. The gonadotropic substance in the serum resembles pure "prolan B" in its luteinizing and synergistic properties. It differs from "prolan B" in that estrus effect was also observed. This latter action, of course, may be due to the presence of a second gonadotropic hormone in blood serum which is effective only when in combination with the synergist.

The marked and consistent gonadotropic effects obtained with the blood serums of normal humans, in the above manner, has led us to investigate the possibility that this reaction may be of value in the study and diagnosis of sex disturbances.

I wish to express my appreciation for the aid and direction of Dr. Samuel Soskin.

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[1] Evans, H. M., Simpson, M. E., and Austin, P. R., *J. Exp. Med.*, 1933, **58**, 561.

[2] Freed, S. C., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 35.

8353 P

# Effect of Administration of Estrogenic Factor upon Hypophyseal Hyperactivity in the Menopause.

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In a previous communication[1] we demonstrated by improved methods[1, 2] the presence of considerable quantities of both follicle stimulating and luteinizing principles or reactions in the blood and urine of menopause patients and castrates.

The present study was undertaken in order to determine whether injection of large quantities of estrogenic substance would influence the secretion and correlated excretion of the gonadotropic factors, as well as to prove or disprove whether such changes actually take place, and whether amelioration of symptoms, as claimed by Sevringhaus,[3] ran parallel to these changes.

Fourteen cases were studied—4 spontaneous menopause, 8 surgical castrates, 2 X-ray menopause.

As in our previous study,[1] fluctuations in excretion were noted in individual women. A long period of preliminary observation was deemed necessary. In 3 cases, this preliminary period was 26, 52, and 63 days. In the other cases, it varied between 2 and 14 days.

From 4,000 to 22,000 R.U. of estrogenic factor (Progynon Benzoate, obtained through the courtesy of Dr. Stragnell of the Schering Corporation) were used. During the preliminary period the daily output of luteinizing factor in the urine varied from an undeterminable quantity to 40 R.U., that of the follicle-stimulating factor from an undeterminable quantity to 56 R.U. In 3 cases in which the gonadotropic factor was determined in the blood, the concentration found during the preliminary study was 25 R.U. per liter.

The estrogenic factor (Progynon B) was administered by intramuscular injection every 2nd or 3rd day until the entire amount had been given.

In 2 cases the gonadotropic factor disappeared from the urine 4 days after the injection and did not reappear for 24 and 26 days

* Joseph Brettauer Research Fellow in Gynecology.

1 Frank, R. T., Salmon, U. J., and Friedman, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

2 Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1236.

3 Sevringhaus, E. L., *J. Am. Med. Assn.*, 1935, **104**, 624.

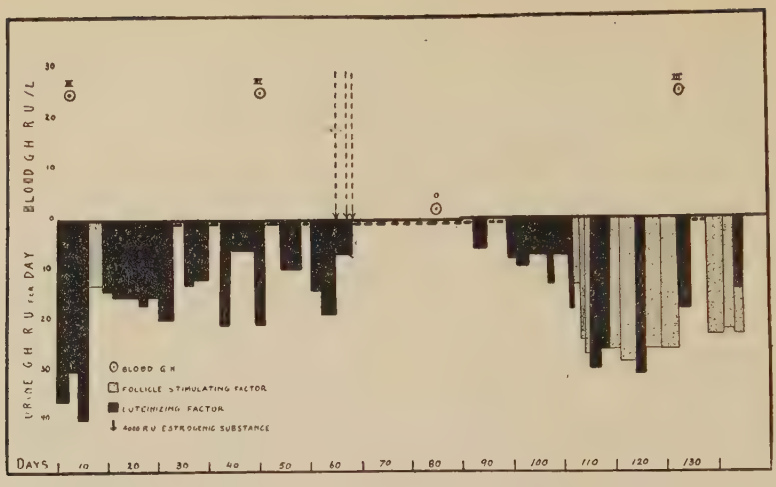


FIG. 1.

*Spontaneous Menopause.* Age 52. 9 years' duration. Each arrow signifies injection of 4,000 R.U. of Progynon B.
G.H.—Gonadotropic factor.
Total duration of observation 135 days.

respectively. In the remaining cases the gonadotropic factor disappeared for 28 to 47 days. In 2 cases the observation was continued for 101 days after the first injection had been given.

*Summary.* In 14 menopause cases in which a hyperexcretion of gonadotropic factor was observed, injection of 4,000 to 22,000 R.U. of estrogenic factor was followed by a rapid decrease of gonadotropic factor in the blood and urine. The disappearance of the prepituitary hormone persisted for from 28 to 70 days but was regularly followed by a return to the condition noted before treatment. The amelioration of subjective symptoms roughly paralleled the decrease of the gonadotropic factor in the urine.

From these studies it appears that the hypophyseal hyperactivity which follows castration or appears in the spontaneous menopause (Zondek,[4] Fluhman[5]) can be temporarily ameliorated by the administration of estrogenic factor in high dosage.

Graph of a typical case is appended.

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[4] Zondek, B., *Klin. Woch.*, 1930, **10**, 2121.

[5] Fluhman, C. F., *Endocrinology*, 1931, **15**, 177.

8354 C

## Elaboration of Hormones by Pituitary Cells Growing in vitro.

EVELYN ANDERSON AND WEBB HAYMAKER. (Introduced by Wilder Penfield.)

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The purpose of this study is to determine whether pituitary cells proliferating *in vitro* elaborate hormones. The pituitary gland was removed from 8-day-old rats and placed in Tyrode's solution. By aid of the dissecting microscope the anterior lobe was separated from the posterior. Each was cut into fragments about 0.2 sq. mm. in size. Anterior lobes of the pituitary were then planted in lots of 5 glands in a Carrel D-3 flask whereas posterior lobes were planted in lots of 10 each. The amount of the various constituents of the medium was as follows: rat plasma, 0.6 cc.; Tyrode's solution, 0.3 cc.; and chick embryonic tissue juice, 0.3 cc. The cultures were washed with salt solution daily and the liquefied areas of the coagulum were patched with 0.1 cc. plasma and 0.1 cc. embryonic tissue juice. The cultures were incubated at a temperature of 38.5°C. and were allowed to grow for 6 days. By that time there was extensive proliferation of epithelial cells of both anterior and posterior lobe explants, an increase of as much as 8 times the original diameter of the explant. On the contrary, there was no evidence of growth of pars nervosa elements.

On the sixth day the coagulum was removed from each flask. That containing the anterior pituitary cultures was broken up in 8 cc. of Tyrode's solution and 1 cc. of absolute ethyl alcohol, added as a preservative. The coagulum containing the posterior lobe cultures was ground in sand. This material was then extracted with 0.25% solution of acetic acid and placed on a boiling water bath for 5 minutes. Both anterior and posterior lobe preparations were stored in the refrigerator at 4°C. and were used within 30 days.

For control material an equivalent number of pituitary glands from rats of the same age were set aside without being cultured. The material was preserved in the same way as that which was cultured.

In assaying the posterior lobe extracted material for hormones the test object for the detection of the melanophore-expanding principle was the frog. For determining the oxytocic and antidiuretic content, the virgin guinea pig uterus and bladder-fistula dog, respectively, were used. Presence of anterior lobe hormones was determined by injection into the hypophysectomized rat.

Only the melanophore-expanding principle was shown to have increased with growth of the posterior lobe *in vitro*. This conclusion was arrived at by comparing the melanophore-expanding effect of cultured posterior lobe material with that of an equivalent number of non-cultured posterior lobes, both treated in a like manner as described above. The frogs were first bleached by being placed in intense light for 40 minutes. The extracted pituitaries in various doses were then injected in the neighborhood of the lymph sac, 8 frogs being used in testing for melanophore-expansion in each of 7 batches. Each batch consisted of a 6-day growth of 10 posterior pituitary glands taken from 8-day-old rats. Of the 8 frogs, 2 received 0.05 unit of pituitrin (Frosst), 2 received the extract of cultured pituitary originating from ¼ of a posterior lobe, 2 received the extract of ¼ of a posterior lobe which had not been cultured and the remaining 2 received no injection. In addition, graded doses of extracts of each lot of cultured pituitary material were injected into a series of 8 frogs in order to determine the minimum effective dose of each lot; this was likewise done with the non-cultured extracted material. The change in the shade of the frogs was noted every 10 minutes for an hour or until the skin of the injected frogs resembled the bleached shade of the non-injected control frogs. The volume of the cultured and non-cultured material injected into the frogs was the same in each case, a sufficient dilution being made with Tyrode's solution to bring it to 0.5 cc.

The results are shown in Table I. The doses tested are expressed in terms of the amount of original pituitary tissue administered per 30 gm. of frog body weight. It was found that 0.05 unit of pituitrin had turned the bleached skin of the frog very dark 40 minutes after injection. When the extract of ¼ of a non-cultured posterior lobe was injected, the melanophore-expanding effect was considerably less (slightly visible, +) than that produced by the injection of an extract of ¼ cultured posterior lobe (very dark, ++++). The latter produced a melanophore-expanding effect roughly equivalent to that of 0.05 unit of pituitrin. The end point (the dilution below which there was no melanophore-expanding effect) in the case of the cultured material was reached with injection of 0.012 of the original lobe whereas the end point with injection of the non-cultured material was 0.10 of the original lobe. Thus, there was approximately an eightfold increase in melanophore-expanding principle with growth *in vitro*.

Subculture of posterior lobe was found to contain melanophore-expanding principle roughly equivalent to that of cultures not trans-

TABLE I.

| Material | Amt. Injected per 30 Gm. of Frog Wt. | Melanophore-Expanding Effect on Skin of Frogs After 40 Min. |
|---|---|---|
| Pituitrin | .05 unit | Very dark, ++++ |
| Non-cultured post. lobe | .25 lobe | Slightly visible, + |
| " " " | .12 " | Very slightly visible |
| " " " | .10 " | " " " |
| " " " | .05 " | No effect |
| Cultured post. lobe | | |
| Lot 40 | .25 lobe | Very dark, ++++ |
| | .10 " | Dark, +++ |
| | .025 " | Slightly visible, + |
| | .012 " | Very slightly visible |
| | .006 " | No effect |
| " 41 | .25 " | Dark, +++ |
| " 42 | .10 " | Very dark, ++++ |
| | .025 " | Slightly visible, + |
| | .012 " | Very slightly visible |
| | .006 " | No effect |
| " 50 | .10 " | Dark, +++ |
| " 50a | .12 " | " ++ |
| " 64 | .08 " | " +++ |
| " 80 | .25 " * | Very dark, ++++ |
| | .10 " | Dark, +++ |
| | .05 " | Slightly visible, + |
| | .012 " | Very slightly visible |
| | .006 " | No effect |
| Subcultured post. lobe | | |
| †Lot 81 (once subcultured) | .50 lobe | Very dark, ++++ |
| | .25 " | Dark, +++ |
| †Lot 82 (twice subcultured) | .50 " | Very dark, ++++ |
| | .25 " | Dark, +++ |
| | .10 " | Slightly visible, + |

*After 100 min. frogs receiving 0.25 posterior lobe of Lot 80 were darker than those receiving 0.05 unit of pituitrin.

†The amount injected is approximately that recorded.

ferred. In transferring the cultures the original explanted fragments of tissue were discarded, so that the transferred material was made up solely of cells which had proliferated *in vitro*. The cultures extracted after one transfer were grown for 9 days; those extracted after 2 transfers were grown 12 days.

In testing of the oxytocic principle the dilution of the extracts appeared to be too great to give a satisfactory test. The test for the antidiuretic principle was more conclusive. An extract containing 1½ posterior lobes, whether cultured or non-cultured, when injected

into a bladder-fistula dog excreting 3.0 cc. of urine per minute resulted in a suppression of urine to 0.4 cc. per minute, the duration of the antidiuretic effect being 25 minutes. Three batches of cultured posterior lobe and 3 of non-cultured were thus tested; results were practically identical.

In regard to the effect of injecting anterior pituitary cultures into hypophysectomized rats, it was found that 60 anterior lobes which had been grown for 6 days, and in which the amount of tissue had increased considerably, produced definite hormonal restorative effects upon the thyroid, adrenals and ovaries of the hypophysectomized rat. This effect, however, was no greater than that produced by an extract of an equivalent number of non-cultured pituitaries. The lack of a more delicate test for assaying anterior lobe hormones was a handicap in this study.

*Conclusion.* With growth *in vitro,* pars intermedia cells of the posterior lobe of the pituitary retain their power to elaborate melanophore-expanding principle. Under the conditions of experiment no discernible production of hormones took place with growth of anterior lobe cell *in vitro.*

We wish to thank Dr. K. I. Melville for his very kind assistance in the assay of posterior lobe hormone.

8355 P

## Effect of Thyroidectomy and Thyroid Feeding on the Estrus Cycle in the Rat.

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Many investigators have reported both anatomical and physiological disturbances in the reproductive system following ablation of the thyroid gland.[1-5] In the adult female rat the most striking functional change is an increase in the length and irregularity of cycles.[4, 5] It seemed of interest to determine to what extent the nor-

[1] Hofmeister, F., *Beitr. zu Klin. Chir.*, 1894, **11**, 441.

[2] Kunde, M., Carlson, A. J., and Proud, T., *Am. J. Physiol.*, 1929, **88**, 747.

[3] Tatum, A. L., *J. Exp. Med.*, 1913, **28**, 500.

[4] Lee, M., *Endocrinology*, 1925, **9**, 410.

[5] Bokelmann, D., and Sheringer, W., *Arch. f. Gynak.*, 1932, **151**, 190.

mal cycles could be restored in thyroidectomized animals by replacement therapy.

Twenty-one young adult rats were thyroidectomized. Vaginal smears were made for varying periods before and after operation and following institution of thyroid feeding (.03 gm. to .06 gm. per day of desiccated sheep thyroid (Armour) added to the food). At the conclusion of the experiment the animals were sacrificed and the neck examined macroscopically for regenerated thyroid tissue. It was found, as in the case of previous workers, that changes in the cycle were not likely to occur if small remnants of thyroid tissue were left behind. In our group 12 animals had had complete extirpation of the gland. These constitute the basis of the present report.

Following thyroidectomy there was generally a single prolonged diestrus period which was attributable to the operative procedure and which was not counted in computing the averages. Complete removal of the gland was followed by a prolongation of the cycle in diestrus averaging one to 5 days. Four to 5-day cycles did occur, but at irregular intervals.

When small doses of desiccated thyroid substance were added to the diet there was a tendency for the cycles to return to normal both in duration and regularity. In most instances, however, the average duration was one day longer than the preoperative level.

When thyroid feeding was discontinued the irregularity and lengthening of the cycles tended to recur.

The number of animals in this series is small. The results, however, indicate a general tendency for alterations in the sexual cycle following thyroidectomy, to be restored toward the normal by replacement therapy with thyroid substance.

Further work is in progress to evaluate the thyroid ovarian relationship.

8356 P

## Carbohydrate Matrix of the Epithelial Cell Inclusion in Trachoma.

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Since the discovery by Prowazek and Halberstaedter of the epithelial cell inclusion in the conjunctiva of patients with trachoma, a voluminous literature has appeared on this structure. The great

majority of the reports have been devoted to affirming or denying that the inclusion body is actually the causative agent of the disease, and little has been done on its composition and nature. The present study, therefore, was undertaken to ascertain certain chemical characteristics of inclusion.

It was found that the inclusion body contains a carbohydrate to a great extent so that when preparations are flooded with Lugol solution, the inclusion gives a sharp color reaction and appears as reddish brown or dark amber against the contrasting yellow background of the epithelial cell. Evidence has been obtained which indicates that the carbohydrate is glycogen, and that it exists in part as a matrix or diffused throughout the inclusion. This evidence consists of (1) preparations of glycogen fixed on a slide with glycerine exhibit similar staining reactions; (2) fading or disappearance of color of iodine-stained inclusions in unfixed preparations on heating, but reappearing with cooling; (3) disappearance or fading of color reaction in 12 to 18 hours in unfixed preparations without reappearing even after restaining with iodine, thus showing solubility of the color reacting substance in water or saline, in contradistinction to amyloid which may give a similar reaction to iodine; (4) rapid fading of the stained inclusion in fixed preparations when treated with concentrated $H_2SO_4$ or HCl, both of which intensify the iodine-stained amyloid; (5) digestion and disappearance of inclusion in fixed smears under the action of saliva; (6) in mountings of epithelial scrapings in very weak iodine solution, the red-brown color of the epithelial inclusions disappears when diluted ammonia water is allowed to pass under the cover slip, just as a glycogen solution in the test tube colored with iodine solution will lose its deep red-brown color on addition of ammonia. The inclusion in unfixed cells stains well with brilliant cresyl blue and carmine red. The inclusion appears to be composed of granules suspended or embedded in the carbohydrate matrix. Some inclusions may contain many more granules than others.

This report will appear *in extenso* in a forthcoming number of the *American Journal of Ophthalmology.*

8357 C

## Pituitary Hormone Antagonism.*

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It is known that certain hypophyseal extracts will inhibit the increase in the size of the ovaries of immature rats, which occurs when gonad stimulating extracts are administered. In a previous note (Leonard[1]), it was stated that the growth hormone of the pituitary was not the inhibiting substance and it was suggested that a sex stimulating fraction or something associated with it, was the antagonistic agent. In further attempts to identify the inhibiting substance with known extracts of the pituitary, several gonad stimulating fractions and a thyroid stimulating fraction were used. The results obtained are reported here.

Antuitrin S† was injected subcutaneously into immature female rats of similar age and weight, in doses which produced ovaries weighing approximately 30 mg., in 5 days. Similar rats were treated for the same period with the same amount of Antuitrin S plus intraperitoneal injections of the several pituitary extracts to be tested for their inhibiting power. These were as follows: follicle-stimulating hormone (F.S.H.) and luteinizing hormone (L.H.) prepared from sheep pituitary (Fevold, *et al.*[2]), luteinizing hormone from horse pituitary, unfractionated pregnant mare serum, follicle stimulating urine (F.S.U.) prepared by alcoholic precipitation, and thyroid stimulator from the Schering Corporation.‡ These preparations were all tested and found to be active.

It is quite expedient that the fraction to be tested for inhibition be given intraperitoneally rather than subcutaneously or otherwise the presence of the inhibiting substance may not be detected (Leonard[1]).

The results of the experiments are summarized in Table I. It is readily seen that the luteinizing fraction prepared from sheep pitui-

* Aided by grants from the National Research Council, Committee on Problems of Sex.

1 Leonard, S. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 1157.

† The Antuitrin S was furnished through the kindness of Parke, Davis and Company.

2 Fevold, H. L., Hisaw, F. L., Hellbaum, A., and Hertz, R., *Am. J. Physiol.*, 1933, **104**, 710.

‡ The thyroid stimulator was obtained from Dr. E. Schwenk, Bloomfield, N. J., through the courtesy of Dr. Philip E. Smith.

TABLE I.

| Treatment | Av. Ovarian Wt. mg. | Av. Uterine Wt. mg. |
|---|---|---|
| Ant. S. (50 R.U.) | 31.9 | 145.2 |
| " + 6.25 mg. L.H. (sheep) | 15.7 | 52.2 |
| Controls (same in all groups) | 15.0 | 38.0 |
| " + 20 mg. preg. mare serum | 30.6 | 114.2 |
| Ant. S. (50 R.U.) | 32.3 | 109.3 |
| " + .2 cc. F.S.H. (sheep) | 22.1 | 81.9 |
| Ant. S. (25 R.U.) | 18.6 | 97.3 |
| " + 100 cc. equiv. of F.S.U. | 46.0 | 83.4 |
| Ant. S. (50 R.U.) | 38.1 | 136.8 |
| " + 6 mg. Thyroid stimulator | 43.6 | 124.3 |
| Ant. S. (75 R.U.) | 40.1 | 146.0 |
| " + 5 mg. L.H. (horse) | 42.1 | 135.7 |
| Ant. S. (75 R.U.) | 33.1 | 109.5 |
| " + .2 cc. F.S.H. (sheep) | 26.9 | 103.8 |

All extracts above were of known potency. .2 cc. of F.S.H. injected subcutaneously gave 25 mg. ovaries; 100 cc. equivalent of F.S.U. subcutaneously gave 26.2 mg. ovaries. The thyroid stimulator tested 1 Rowland-Parkes unit per 1.5 mg.

Each result is based on the average of 3 or more rats. All fractions tested for inhibition (excluding Ant. S) were injected intraperitoneally.

All rats were 22-25 days old at the beginning and were autopsied 5 days later.

tary was the only one to give a complete and definite inhibition of ovarian growth. The follicle stimulating substances from pituitary or urinary sources failed; the pregnant mare serum, the thyroid stimulator and the luteinizing hormone from horse pituitary also failed. It is noted that when F.S.U. was injected, there was a decided increase in ovarian weight over that produced by the Antuitrin S alone. This may have been due to some of the F.S.U. entering the subcutaneous tissue which would give an "Augmented" reaction with Antuitrin S.

It was again observed that whenever the inhibiting action is complete, the uterus of the immature rat fails to grow and the vagina does not open. In these experiments only the L.H. fraction from sheep pituitary inhibited uterine growth and vaginal canalization.

From the results in Table I, it is not possible to conclude that the luteinizing hormone *per se* is the inhibiting substance. The luteinizing fraction from horse pituitary did not inhibit the gonad stimulating action of Antuitrin S. Other experiments have also showed that L.H. from horse pituitary will not inhibit the pituitary follicle stimulator when injected in the same way.

It can only be concluded from these results that the inhibiting

principle is associated with the luteinizing hormone of certain pituitary glands. At present, the reaction itself can only be demonstrated in the immature and hypophysectomized rat. Attempts to inhibit the stimulation of adult rat ovaries by the injection of Antuitrin S and L.H. from sheep pituitaries were unsuccessful.

8358

## Negative Results of Iodides in Anaphylaxis in Guinea Pigs.

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Zifferblatt and Seelaus[1] have reported successful desensitization of guinea pigs by repeated intraperitoneal injection of an aqueous solution of iodine. This report would appear to lend support to the belief long held by many clinicians that in some asthmatics continued iodide administration rendered their attacks less frequent and severe. It, therefore, seemed worth while to see if iodide therapy in sensitized guinea pigs would also result in desensitization. The negative results obtained are stated in Table I.

All pigs received a subcutaneous injection of 1/20 ccm. of dog serum January 11.

TABLE I.

| Treatment | ½ ccm. dog serum I.V. | Result |
|---|---|---|
| None | Feb. 23 | died |
| " | Mar. 2 | lived |
| " | " 6 | died |
| 100 mg. Iodalbumin | Feb. 23 | lived |
| (21.5% I) daily from | " 23 | died |
| Feb. 11 | Mar. 2 | " |
| | " 2 | " |
| | " 6 | " |
| | " 6 | " |
| 25 mg. K.I. I.P. | Feb. 23 | " |
| daily from Feb. 11 | Mar. 6 | " |

All pigs were autopsied and in those dying after the intravenous injections, the lungs showed typical distension.

Although the series is small the results are so negative that it may

[1] Zifferblatt and Seelaus, *Am. J. Med. Sci.*, 1934, **188**, 142.

be concluded that iodide administration does not desensitize sensitized guinea pigs.